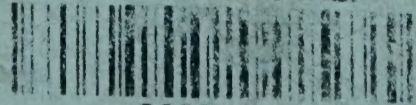


# *The Avian Embryo*

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morphogenesis, ~~Heart~~, Heart,  
Nervous system, urogenital system,  
Sense organs, Digestive system,  
Blood vessels, endocrine glands,  
~~Extra~~embryonic membranes,

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# The Avian Embryo

*Structural and Functional Development*





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# The Avian Embryo

## Structural and Functional Development

By

ALEXIS L. ROMANOFF

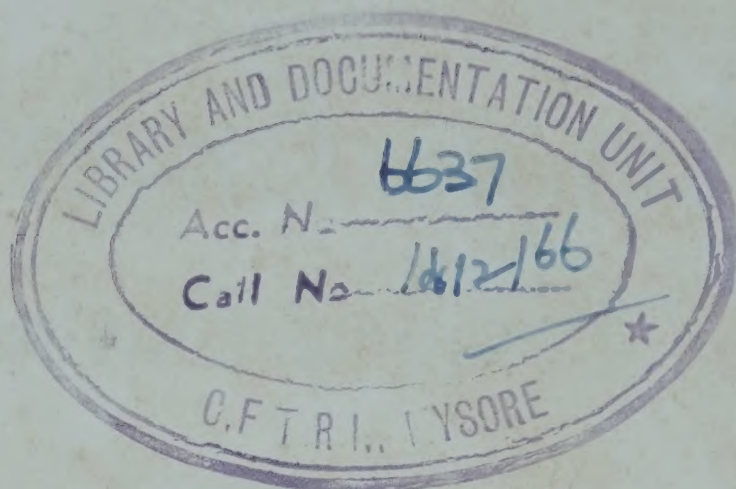
*Professor of Chemical Embryology  
Cornell University*

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Avian embryo: St.



Dedicated

to

*the Scientists throughout the World  
whose Search for Truth Furthered the Knowledge  
of Avian Embryology*







## PREFACE

This book, *The Avian Embryo*, is a complementary and companion volume to *The Avian Egg*, published in 1949. It is an effort to bring together, in one volume, all the known scientific facts about the structural and functional development of the avian embryo.

My chief objective is to analyze the existing knowledge of this subject which has been scattered throughout the world literature, published in many languages, and was frequently inaccessible to most scientists. I have aimed to have this volume suitable both as a text for advanced students and teachers in zoology and comparative embryology and as a reference work for scientists, especially research workers in medico-biological and numerous other fields in which the avian embryo is used as an experimental material.

In the preparation of this book, it was necessary to make a critical study of more than 7,000 original publications, about two-thirds of which are written in languages other than English. Of these only about 2,700 are cited in the text. The combined bibliography is arranged alphabetically according to the authors' names, with page references, in order to facilitate the locating of further details in the original literature. The text is supplemented by 441 illustrations consisting of 2,256 pen-and-ink drawings, which have been derived from either various published sources or my unpublished material. The inclusion of these graphic compositions, it was thought, would give a clearer understanding of some rather involved developmental processes and shorten the discussion demanded by the various topics. A detailed subject index is included in place of a complete outline of the table of contents, thus providing a more useful means of quick orientation in finding answers to specific inquiries.

The present volume, an anatomicophysiological study, is but a part of my lifelong plans to review and assess the entire factual knowledge of avian embryology, including the chemical and pathological aspects. However, the exceedingly rapid increase of scientific literature today, it seems, would have required extraordinarily favorable conditions, besides one's personal knowledge, experience, and tenacity, to complete this almost unsurmountable task within one's allotted time.

It is hoped, nevertheless, that the completed portions, *The Avian Egg* and the present book, *The Avian Embryo*, may serve not only as a source



of information for inquiring minds, but also as a starting point for an experimental search for new facts leading to discoveries of some still missing links in our knowledge of prenatal development.

ALEXIS L. ROMANOFF

ITHACA, NEW YORK



## ACKNOWLEDGMENT

There is no adequate way for the author to express his gratitude to all those who have assisted him in various capacities, at various times during the many years of preparation and the ten years of writing this book. He is especially grateful to his former students for their frank criticism; to his professional colleagues for their generous responses to his requests for information; to certain senior university scholars for their wise and constructive suggestions; to scientists throughout the world for their co-operation in providing some published material heretofore unknown elsewhere; and finally, to numerous friends for their unfailing encouragement in this undertaking.

Individually, the author extends his appreciation to his wife, Anastasia J. Romanoff, co-author of *The Avian Egg*, for her collaborative experimental and library research, and her deepest interest and inspirational insight into the entire project from the time of its inception.

The author is thankful to Dr. Ernst Mayr, of the Museum of Comparative Zoology, Harvard University, for his advice on the scientific names of birds; and to Dr. Glenn V. Russell, of the University of Texas Medical School, for his critical reading of the chapter on the nervous system.

The author also extends his appreciation to Muriel C. Scolnik, Carlita L. Nesslinger, Regina E. Giordano, Frances E. Sage, and Ella S. Kellogg for their assistance in preparing the text; and to Janet A. Perry and Frances A. McKittrick for their help with some of the illustrations.

The completion of this work would not have been possible without the traditionally free academic atmosphere of Cornell University, with which the author has been for many years happily affiliated; as well as generous assistance from personnel of the Library of Congress, of the Army Medical Library, of the Library of the New York Academy of Medicine, and of Cornell University with its various college and department libraries. And especially the author gratefully acknowledges his appreciation to Dr. J.H. Bruckner, the Head of the Department of Poultry Husbandry, Cornell University, for providing a most comfortable environment, and for his sympathetic understanding.







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# *The Avian Embryo*

*Structural and Functional Development*







# INTRODUCTORY NOTE

From the very beginning of our knowledge of embryology, the avian embryo has occupied a unique position among the higher vertebrates. It has an extra-uterine existence. All its requirements, except oxygen, are provided within the egg, which is by far the most self-contained organism, and is capable of developing in a terrestrial environment. Also, because of its universal availability, it undoubtedly has been an object of embryological observations from earliest historical time.

## A Brief History of Avian Embryology

Presumably some elementary understanding of embryology of birds goes back to antiquity. From the fragmentary records available, there is some evidence that it was known to ancient Egyptians, Babylonians, Assyrians, and Indians, and also to Chinese.

The first fully preserved account, a detailed description of the chick embryo, is credited to Aristotle (384–322 B.C.), a Greek philosopher, in his *Historia Animalium*. A somewhat less accurate description was given by Pliny, the Elder (23–79 A.D.), a Roman scholar, in his *Historia Naturalis*. And then, many centuries later, in the Renaissance period, an Italian naturalist, Ulisse Aldrovandi (1522–1605), in his *Opera Omnia*, described the early stages of chick development. Almost at the same time, Fabricius ab Aquapendente (1537–1619), the Italian anatomist and surgeon, made further observations in his *De Formatione Ovo et Pulli*. A generation later, Marcello Malpighi (1628–1694), the Italian anatomist, laid the foundation for modern understanding of avian embryology through his published works, *De Formatione Pulli in Ovo* and *De Ovo Incubato*. This promising beginning of avian embryology was pursued by Albrecht Haller (1708–1777), the German physician, whose notable observations on the chick were published in his *Elementa Physiologiae Corporis Humani*. These initial efforts were followed by many more studies of embryology by highly capable men throughout Europe.

During the nineteenth century, the science of embryology was firmly established by research, more of which was performed on the avian embryo than on any other single animal. For many years, however, the study of avian embryology was largely confined to the classroom and was of little interest except to small specialized groups of scientists. At first, these sci-



entists concerned themselves chiefly with descriptive morphology and to some extent with physiology. The results of their work appeared in some notable studies, among which must be mentioned a stimulating book, *Specielle Physiologie des Embryo* (1885), by W. Preyer, and a monumental work of its time on comparative embryology, *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere* (1901–1906), in six volumes under the editorship of Oskar Hertwig. Soon after, there appeared a small, classical, elementary book on avian embryology in the English language, *The Development of the Chick* (1908) by Frank R. Lillie, who was, it seems, greatly influenced by the impact of Hertwig's well-documented summary in German.

With the beginning of the twentieth century, investigators in embryology turned their attention increasingly to the chemical and physical phenomena associated with embryonic development. Perhaps the most elucidative analysis of experimental results in comparative embryology, with great emphasis on the avian, was made by Joseph Needham in his *Chemical Embryology* (1931), in three volumes. But since then, the ever-increasing output of scientific data by the rapidly growing number of investigators has proved the avian embryo to be an exceptionally suitable animal for experimentation.

### Avian Embryology Today

Today, research on the avian embryo is a means to an end, as well as an end in itself. Developing birds' eggs are now widely used by workers in both pure and applied sciences and are of interest not only to embryologists and educators but also to biochemists, pathologists, geneticists, agricultural scientists, and a host of others. The incubating egg has become standard experimental material in the study of nutritional and endocrine deficiencies, disease transmission, viruses, cancers, and varied kindred medical problems, and is well known as a source of vaccines and a testing ground for pharmaceuticals. In addition, it offers an opportunity for the investigation of hitherto little explored questions, such as the role played by extrinsic, environmental factors in the production of abnormalities.

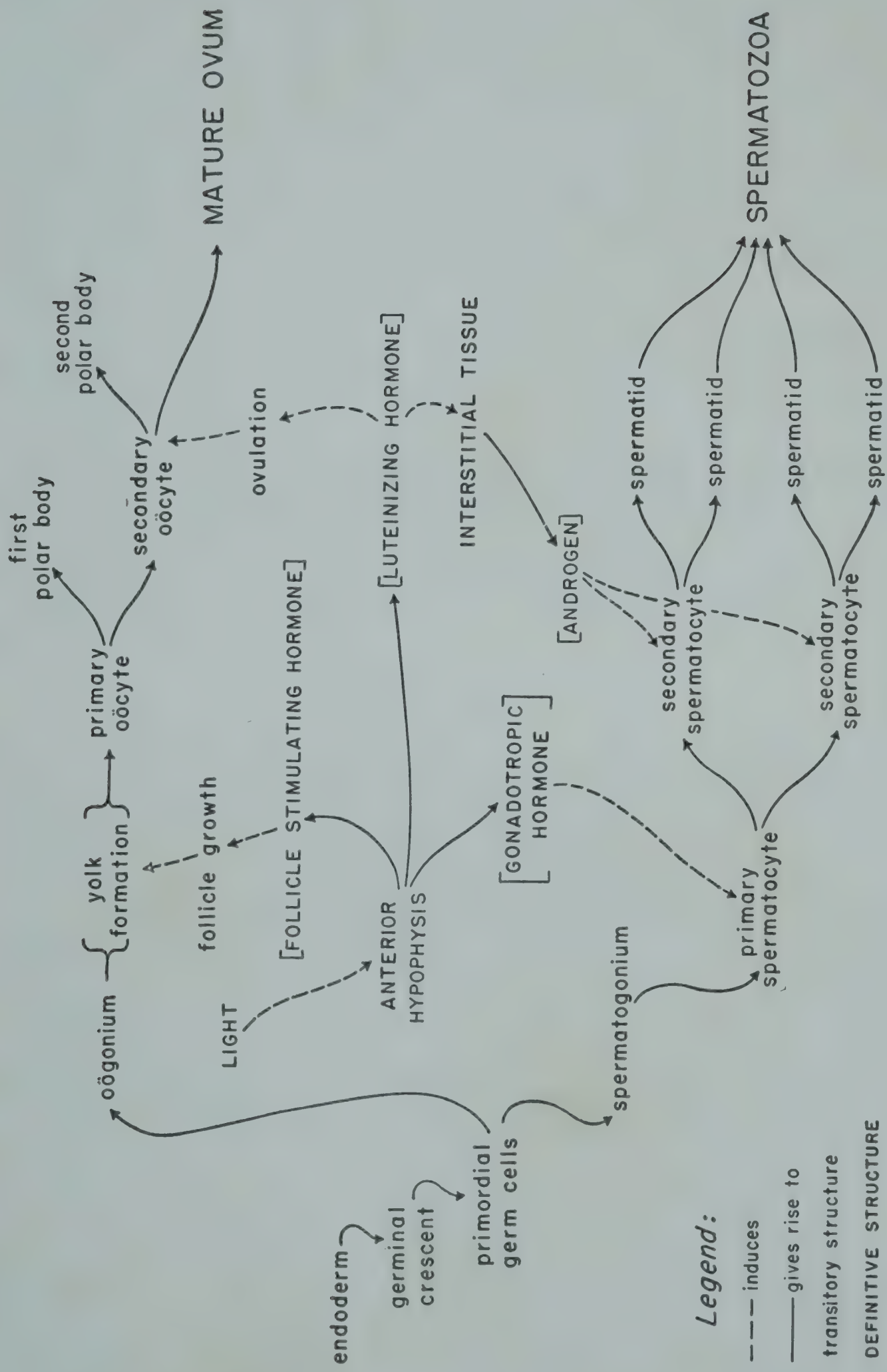


## CHAPTER ONE

# *The Reproductive Cells*

*Within the innate constitution  
Of parents' reproductive cells,  
There is a hidden contribution  
To living creatures in their shells.*





## DEVELOPMENT OF THE REPRODUCTIVE CELLS IN AVES

# THE REPRODUCTIVE CELLS

The individual bird embryo comes into existence as the result of the union of two germ cells, or gametes. These are the ovum, or egg, produced by the female parent, and the spermatozoon, originating in the body of the male parent. When the nuclei of the germ cells combine, the ovum is said to be fertilized. Its protoplasmic components are now complete, the conditions necessary for normal cell division are fulfilled, and the development of the embryo soon proceeds.

Logically, then, a study of the avian embryo starts with a description of the antecedent germ cells. By the time fertilization occurs, both the ovum and spermatozoon have already undergone a succession of nuclear and cytoplasmic changes. In order to relate the complete history of the reproductive cells, it is necessary to return to a period early in embryonic life.

## THE PRIMORDIAL GERM CELLS

The earliest embryonic ancestors of the mature sex cells of the adult are known as primordial germ cells. It appears that these cells are not originally present in the gonads but migrate to their definitive position early in the developmental period. Subsequently, they undergo the process of maturation (spermatogenesis or oögenesis) which prepares them for their specific function.

### The Origin and Migration of the Primordial Germ Cells

The primordial germ cells are segregated from the somatic cells in the very young (primitive streak) embryo. In the chick (*Gallus gallus*) embryo, they can be recognized after about 18 hours' incubation, when they are situated anterior and anterolateral to the embryo proper, along the boundary between the area pellucida and the area opaca. These areas are the inner and outer zones, respectively, of the blastoderm, which is the living, cellular portion of the egg (see Chapter 3). The region occupied by the primordial germ cells is often called the "germinal crescent" because of its form when viewed from above. The concavity of the crescent is turned posteriorly, toward the embryo (Fig. 1-A). According to Rawles (1936) and Willier (1937b), the lateral arms of the germinal crescent have a considerable



posterior extent. A few germ cells have been found medial to the germinal crescent.

Cross sections of the blastoderm show that the germ cells lie free in the space between the ectoderm and the endoderm (the uppermost and the innermost of the three primitive layers of embryonic tissue). It has been said that they differentiate and bud off from the germ wall endoderm (Fig. 1-B).

When the primordial germ cells can first be identified, the avian embryo has attained a stage of development considerably more advanced than that at which reproductive cells have been recognized in certain lower forms of

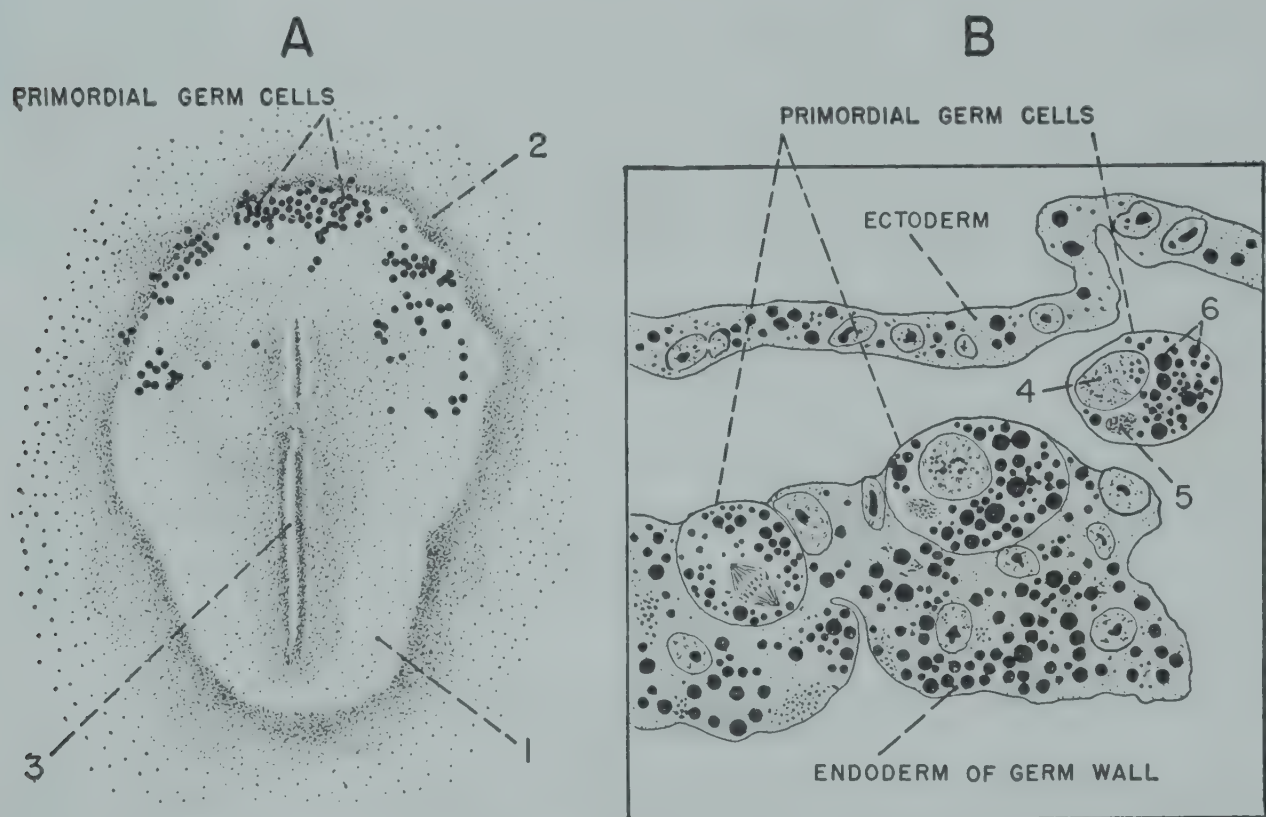


Fig. 1. The location of the primordial germ cells of the chicken (*Gallus gallus*) at the time they first become identifiable in the developing embryo. (A, reconstructed from Swift, 1914, and Willier, 1937b; B, redrawn from Swift, 1914.)

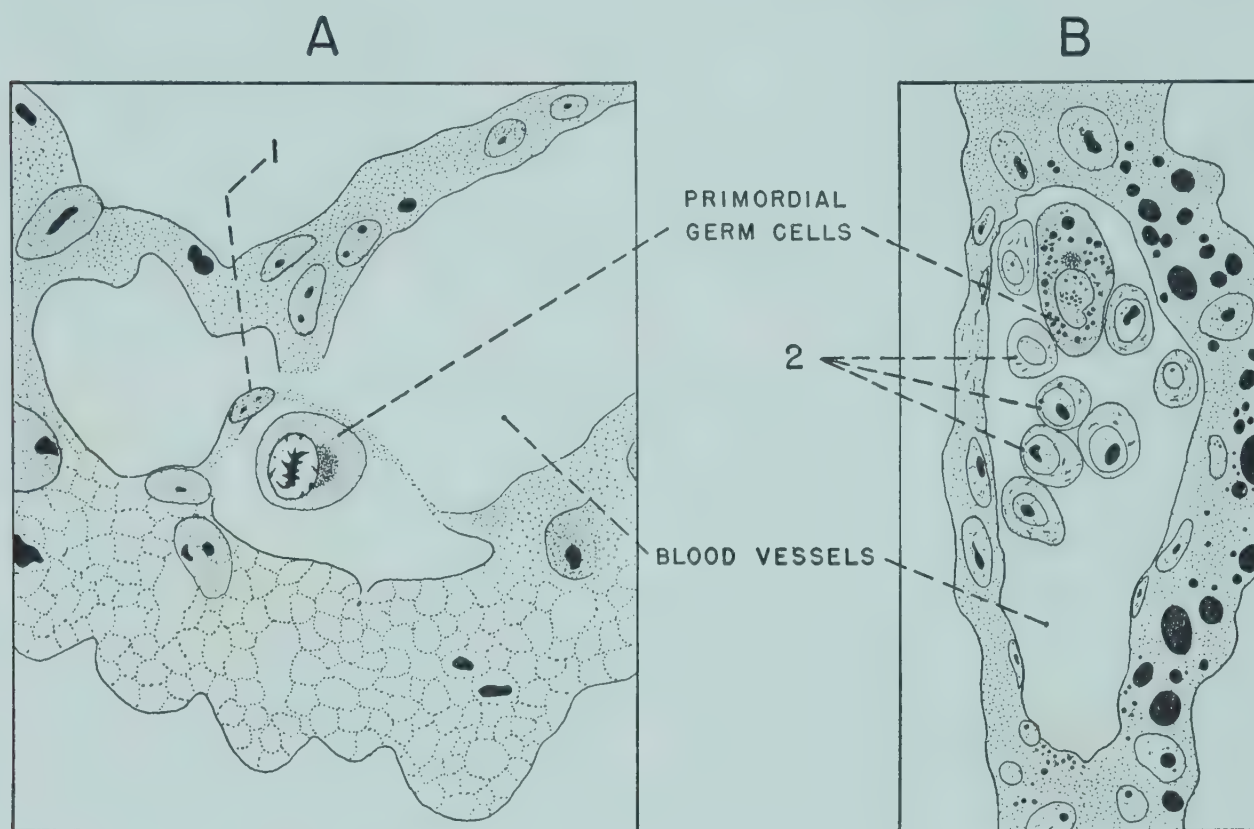
A, surface view of the blastoderm in an egg incubated 18 hours, showing the germ cells in the "germinal crescent" ( $\times 20$ ); B, portion of a section through the edge of the area opaca in the region of the germinal crescent ( $\times 500$ ).

1, area pellucida; 2, area opaca; 3, primitive streak; 4, nucleus of primordial germ cell; 5, attraction sphere; 6, yolk spherule.

life. In *Ascaris*, for example, Boveri (1892) was able to demonstrate the descent of the gametes from one of the two cells produced by the first division of the fertilized egg, thereby virtually proving that the Weismannian concept of the continuity of germ plasm is valid, at least for this genus. In *Aves*, an unbroken line of protoplasm extending from generation to generation has not been demonstrated; no evidence of germ cell differentiation during the early cleavage of the bird's ovum has as yet been presented. The 10-hour chick embryo is the youngest in which primordial germ cells have allegedly been seen. Their presence in the posterior part of the primitive

streak (the forerunner of the embryonic body) was reported by Matsumoto (1932), who believed that they arise at the posterior margin of the blastoderm, rather than anteriorly.

From the germinal crescent, the primordial germ cells make their way to their ultimate destination, the region where the gonads will develop. As the cells of the mesoderm (the third primitive layer of embryonic tissue) grow forward between the ectoderm and the endoderm, they surround the germ cells. Within the mesoderm, the blood islands—forerunners of blood vessels—begin to differentiate. The germ cells are soon found mingled with blood cells in newly formed vessels, into which they have either been in-



**Fig. 2.** Primordial germ cells in blood vessels of the avian embryo. (Redrawn with modifications A, from Blocker, 1933; B, from Swift, 1914.)

A, a primordial germ cell entering a blood vessel in a 10-somite sparrow (*Passer domesticus*) embryo; B, a primordial germ cell in a blood vessel of a 19-somite chicken (*Gallus gallus*) embryo. Both  $\times 700$ .

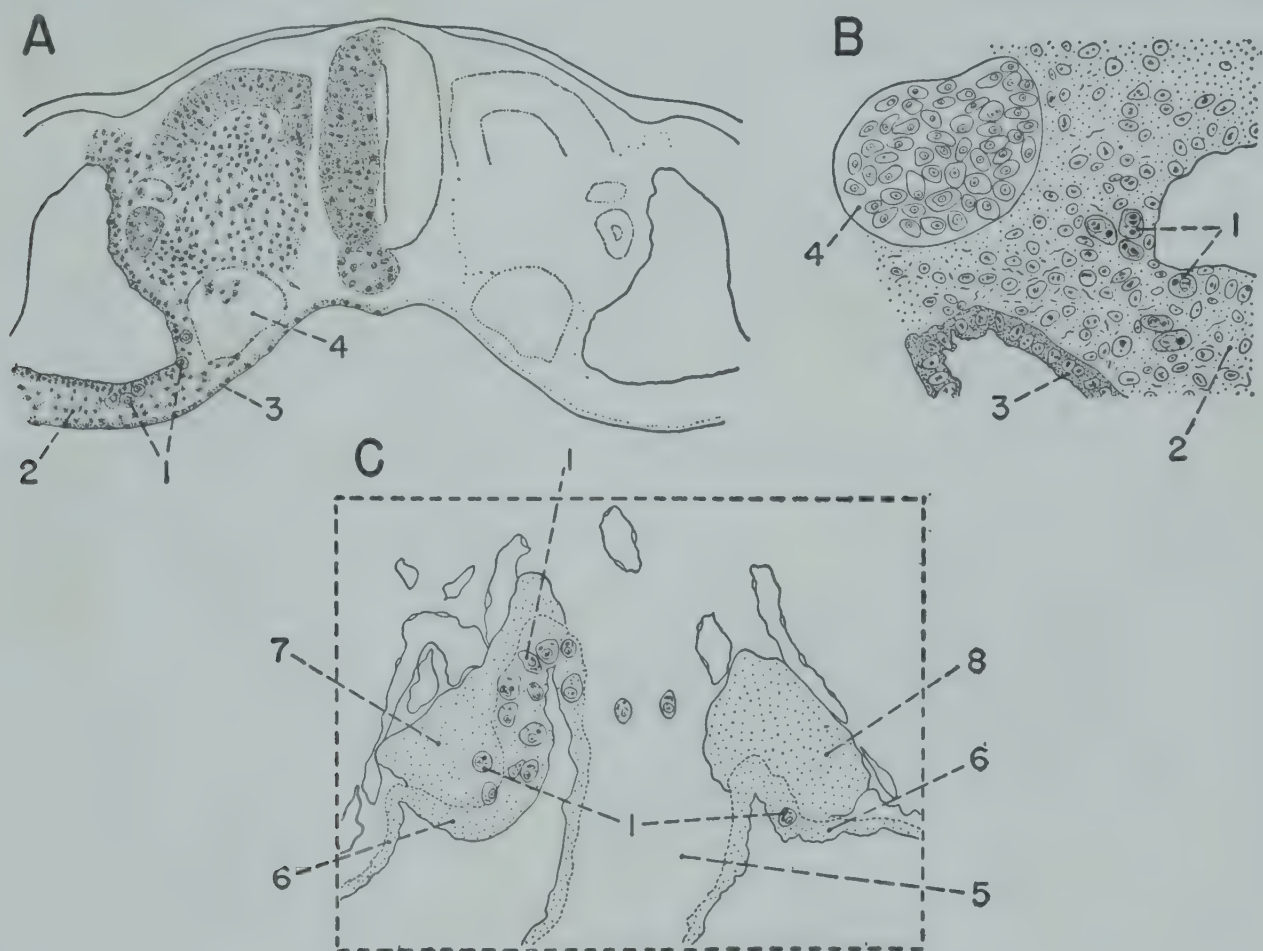
1, endothelial cell being forced aside by a primordial germ cell; 2, blood cells.

corporated passively, migrated by amoeboid movement, or forced themselves by mechanical pressure (Blocker, 1933). Figure 2-A shows a primordial germ cell pushing aside a cell of the endothelial lining of a blood vessel, and Fig. 2-B shows a primordial germ cell mingled with blood cells in the lumen of a blood vessel.

After about 33 hours of incubation, the embryonic and extraembryonic circulatory systems of the chick embryo become continuous, and the germ cells enter the body of the embryo. They are now circulated freely and have been found in such incongruous places as the heart and the head (Swift, 1914). About 10 hours later (in the 21- to 25-somite embryo), they have collected in the blood vessels of the splanchnic mesoderm, closer to the site



of the future gonads. In the opinion of Swift (1914), Firket (1914), and Reagan (1916), it is the operation of some type of chemotaxis that causes the germ cells to gravitate toward this region. Blocker (1933) and Danchakoff (1931), however, have noted that the germ cells, which are large, become lodged in the small capillaries in the posterior part of the embryo where the circulation is slow and the pressure low; and these observers therefore concluded that a purely mechanical process arrests the germ cells in the proper vicinity.



**Fig. 3.** Final stages in the migration of the primordial germ cells in the chick embryo. (Redrawn with modifications A, from Rubaschkin, 1907; B, from Swift, 1914; C, from Swift, 1915.)

A, primordial germ cells in the splanchnic mesoderm and coelomic angle of the 29-somite embryo ( $\times 40$ ); B, primordial germ cells at the coelomic angle of the 33-somite embryo ( $\times 140$ ); C, unequal distribution of primordial germ cells on the right and left sides of a 4-day embryo ( $\times 100$ ).

1, primordial germ cell; 2, splanchnic mesoderm; 3, endoderm; 4, aorta; 5, mesentery; 6, germinal epithelium; 7, left gonad; 8, right gonad.

Within a very short time, the germ cells disappear from the blood vessels, apparently passing through the walls of the capillaries by amoeboid movement. Continuing to progress by this means, they migrate in a dorsal and a lateral direction on either side of the embryo's body. In the 33-somite chick embryo, they are found passing through the splanchnic mesoderm (Fig. 3-A) and around the coelomic angle into the root of the mesentery and the somatic mesoderm (Fig. 3-B). Eventually, they attain the coelomic epithelium. Here some of them push their way between the epithelial cells,



which they compress and deform. Other primordial germ cells remain in the mesenchyme underlying the epithelium. The primordia of the gonads, found on the median surface of the Wolffian bodies (primitive kidneys) in the 4.5-day chick embryo, are formed, therefore, of epithelium and underlying stroma both of which contain primordial germ cells (Fig. 3-C).

The germ cells distribute themselves very unequally between the two sides of the embryo, especially in the female. Even before the differentiation of sex (cf. Fig. 3-C), the germ cells collect on the left side in numbers from two to five times as great as on the right (*Firket, 1913, 1914; Swift, 1915; Defretin, 1924; Blocker, 1933*). In fact, an active migration of the germ cells from the right side to the left has been observed, not only in the chick (*Firket, 1914; Swift, 1915*), but also in such birds as the English sparrow (*Passer d. domesticus*) and the red-winged blackbird, *Agelaius phoeniceus* (*Witschi, 1935b*). By the time the female chick is 3 weeks old, few if any germ cells remain in the right gonad (*Brode, 1928*). In adult male birds, the disparity between right and left remains, the left testicle being the larger (*Etzold, 1891*).

Danchakoff (1931), who found poorer development of the embryonic arterial network on the right side, ascribed the bilateral asymmetry of the gonads to vascular factors. Early writers advanced the theory of chemotaxis in explanation of the phenomenon. This theory has been reinterpreted somewhat by more recent investigators, who have suggested that the cortex of the gonad exerts an attraction for the germ cells. Since the right ovary fails to develop a cortex, a congenital deficiency of the cortical inductor of gonad differentiation on the right is perhaps responsible for the asymmetrical distribution of the germ cells in the female (*Witschi, 1935b*). According to another view, the inherently superior developmental capacity of the left side of the blastoderm (*Rawles, 1936*) establishes a primary ratio in favor of the left side before the germ cells start to migrate from right to left. In some hawks—Cooper's hawk (*Accipiter cooperi*) and the marsh hawk (*Circus hudsonius*)—this migration apparently does not occur, yet the left gonad of the embryo nevertheless is endowed with more germ cells than the right (*Stanley and Witschi, 1940*).

Having attained the gonads, the germ cells become intimately involved in the subsequent development of these organs. This process will be described in Chapter 10.

It should be remarked at this time that the probable extraembryonic origin of avian germ cells and their migration into the gonads remained unsuspected for many years. In 1870, Waldeyer observed primordial germ cells in the 4-day chick embryo at the site of the developing gonad, where they were mingled with epithelial cells. It was logical for him to conclude that the epithelial cells differentiated and gave rise to the definitive germ cells; hence, the epithelium covering the gonad came to be known as the



germinal epithelium. He did not find any transitional forms between epithelial cells and sex cells, but other investigators studying avian material subsequently claimed to have done so (Semon, 1887; Hollander, 1904; Essenberg and Garwacki, 1938).

About 20 years after Waldeyer published his findings, Hoffmann (1892) observed germ cells in embryos of the oyster catcher (*Haematopus ostralegus*), the tern (*Sterna* sp.), and the gallinule (*Gallinula chloropus galeata*) at the 23-somite stage, which is a period in development antedating the formation of the germinal epithelium. The cells were present in the splanchnic mesoderm, in the endoderm, and in the region between the two, and thus were considerably removed from the actual location of the future sex glands. Nussbaum (1901c), Tschaschin (1910b), and Berenberg-Gossler (1912), working with the chick embryo, and Rubaschkin (1907), working with both the chick and the duck (*Anas platyrhynchos*) embryo, corroborated Hoffmann, although Berenberg-Gossler (1914) later ascribed a somatic character to the primordial germ cells. Danchakoff (1908b) noted the presence of primordial germ cells in the blood stream and correctly described their origin, but she did not identify them or follow them after their disappearance from the circulation. It remained for Swift (1914) to present their history as it has been given above.

To test the validity of Swift's findings, Reagan (1916) excised the germinal crescent from early embryos and discovered that the gonads of the operated embryos contained no germ cells at the end of 4 or 5 days of incubation. Similar results were obtained by Benoit (1930), who used ultraviolet irradiation to destroy the germinal crescent, and by Danchakoff, Danchakoff, and Bereskina (1931), who employed electric cautery for the same purpose. On the other hand, Essenberg and Svejda (1939) duplicated these experiments and found germ cells in the gonads of all embryos that survived until the sixth incubation day.

The extraregional origin of the primordial germ cells is now fairly well established. Nevertheless, there has been some reluctance to accept these early cells as the sole ancestors of the adult gametes. Firket (1914) contended that most of the primordial germ cells degenerate before the hatching date and that the definitive sex cells then differentiate from the germinal epithelium. He admitted, however, that some of the primordial cells might possibly survive and give rise to definitive cells. Essenberg and Garwacki (1938), who agreed essentially with Firket, stated that fully formed germ cells are present in the germinal epithelium before blood circulation is established, a circumstance which, if true, rules out the descent of the germ cells from primordial cells brought to the gonads by the blood stream. Formation of germ cells from peritoneal cells in hens (*Gallus gallus*) undergoing sex reversal has been reported by Fell (1923) and by Gatenby (1924). Hooker and Cunningham (1938), who described several cases of



apparent retroperitoneal regeneration of the testis in castrated cocks, suggested that there may be two seats of origin of the germ cells in birds, one of them purely somatic.

Goldsmith (1928), however, gave a convincing account of the descent of mature ova and spermatozoa in an unbroken line from primordial germ cells. Neither degeneration of the primordial germ cells nor the arising of a new generation of sex cells from somatic elements could be detected by Blocker (1933) in the English sparrow (*Passer d. domesticus*) embryo. Furthermore, Domm (1929) demonstrated the failure of germ cells to form anew in the chick's right ovary (which normally retrogresses) if the left ovary is removed after the disappearance of germ cells from the right ovary. If the left ovary is removed prior to their disappearance from the right ovary, the germ cells already present in the right ovary may develop into spermatozoa (Benoit, 1923). This versatility supports the viewpoint of Willier (1937b, 1950), namely, that the primordial germ cells are not true sex cells, but cells of a generalized nature, capable of differentiating in either direction—male or female—according to the specific sexual environment provided by the gonad. Except for their lack of sexual specificity, the primordial germ cells should be regarded as reproductive cells, the preponderance of the evidence indicating that they give rise to the definitive sex cells.

### The Structure of the Primordial Germ Cells

The primordial germ cells are large, turgid, and usually round to ovoid (Fig. 4-A), although pseudopodia frequently distort their shape. Their distinct outline and well-demarcated nucleus differentiate them from the somatic cells, but they stand out chiefly because of their great size. According to Swift (1914) and Danchakoff (1931), their diameter ranges from 14 or 15 microns to 22 microns in the chick embryo, although it does not often exceed 18 microns. Goldsmith (1928) gave their diameter as 10 to 15 microns.

The nucleus alone is larger than an entire somatic cell (Goldsmith, 1928). Swift (1914) stated that it measures 8 to 10 microns in diameter before the germ cells leave the germinal crescent and 10 to 12 microns afterward. The nucleus is spherical, eccentrically placed, and apparently surrounded by a definite membrane. It contains proportionately less chromatin than the nucleus of a somatic cell. This material is at first scattered more or less evenly throughout the nucleus, often in a reticular pattern; later it gathers into two irregular, granular masses separated by a variable distance. Sometimes these masses are connected by granular threads (Swift, 1914), which, in the English sparrow, *Passer d. domesticus* (Blocker, 1933), may appear as radiating strands. There is no sharply defined nucleolus. Swift (1914) and Blocker (1933) observed no mitotic figures



in the migratory germ cells, but Danchakoff (1931) found that such figures are always present, though not numerous.

The cytoplasm contains a prominent centrosphere or attraction sphere (cf. Fig. 4-A) which, in the chick (*Gallus gallus*), measures from 3 to 4 microns in width and up to 6 microns in length (Swift, 1914). This structure is found on the side of the eccentric nucleus which is farthest removed from the cell membrane. Sometimes the attraction sphere is closely applied to the nuclear membrane, and at other times a space intervenes between them. Within the attraction sphere there are two central bodies, or centrioles. Within the attraction sphere there are two central bodies, or centrioles.

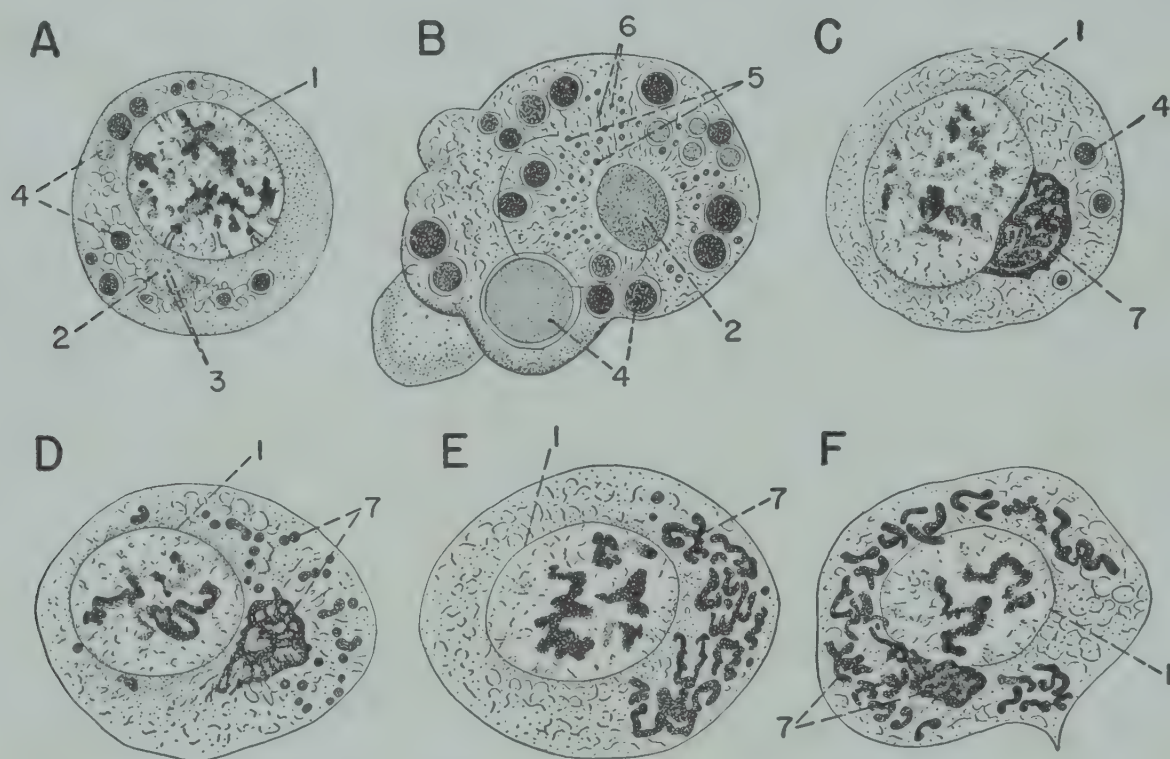


Fig. 4. Structure of the primordial germ cells of the chick embryo. (Redrawn with modifications from Danchakoff, 1931.)

A, primordial germ cell from a 2.5-day chick embryo (Zenker-iron hematoxylin technique); B, primordial germ cell sectioned in such a way that the nucleus is not transected and the radiation of mitochondria and cytoplasmic trabeculae from the centrosphere is clearly apparent (Champy-Altmann technique); C, D, E, F, various forms assumed by the Golgi apparatus in primordial germ cells found in a 3-day embryo (Kolatscheff-safranin technique). All  $\times 2250$ .

1, nucleus; 2, centrosphere; 3, centrioles; 4, yolk sphere; 5, mitochondria; 6, cytoplasmic trabeculae; 7, elements of Golgi apparatus.

Woodger (1925) could not demonstrate the Golgi apparatus in the germ cells before their entrance into the blood vessels. Thereafter, the Golgi bodies are grouped around the attraction sphere. The Golgi apparatus varies considerably in appearance, as Danchakoff (1931) remarked when studying the 3-day chick embryo (Fig. 4-C, D, E, and F). The bodies may be closely or loosely knit, or they may be scattered in the cytoplasm, sometimes almost surrounding the nucleus. Small, round Golgi bodies are found only in cells where there are no yolk spheres and may be concerned, therefore, with yolk resorption (Danchakoff, 1931).



The mitochondria are described as granules (Fig. 4-B) by Danchakoff (1931) and Tschaschin (1910*a*, 1910*b*). Swift (1914) saw them as beaded or granular rods (chondrioconts) 1 to 3 microns long, more or less evenly distributed throughout the cytoplasm.

Yolk, in the form of spheres, persists in the germ cells long after it has disappeared from the somatic cells. It is first found massed in the region of the attraction sphere, sometimes even extending around the nucleus (cf. Fig. 4-A and B). By the time the germ cells reach the gonad primordia, the amount of yolk has decreased to five or ten spherules situated between the attraction sphere and the cell wall (Swift, 1914).

Having arrived in the gonads, which soon begin to differentiate as male or female sex glands, the germ cells multiply rapidly. In the female chick embryo, this period of cell division takes place between the eighth and eleventh days, and in the male it begins on the thirteenth (Swift, 1916) or the fifteenth day (Goldsmith, 1928). At this point, the male and female germ cells diverge in development, their evolution being known as spermatogenesis in the male and as oögenesis in the female. Oögenesis is the more complex process. It is particularly so in birds, whose eggs are laden with relatively enormous quantities of yolk.

## OÖGENESIS

The bird's egg is characterized by the presence of a great mass of non-living material organized according to a definite structural pattern. This portion of the egg, consisting of the yolk substance, the albumen, and the shell with its membranes, serves the double function of nourishing and protecting the developing embryo. The germ plasm, microscopic in amount, is found in a circumscribed region on the surface of the yolk, which alone of the macroscopic components of the egg is produced by the ovary. The yolk, in fact, is the ovum. The nuclear and cytoplasmic changes preceding the ovulation of a mature ovum are intimately correlated.

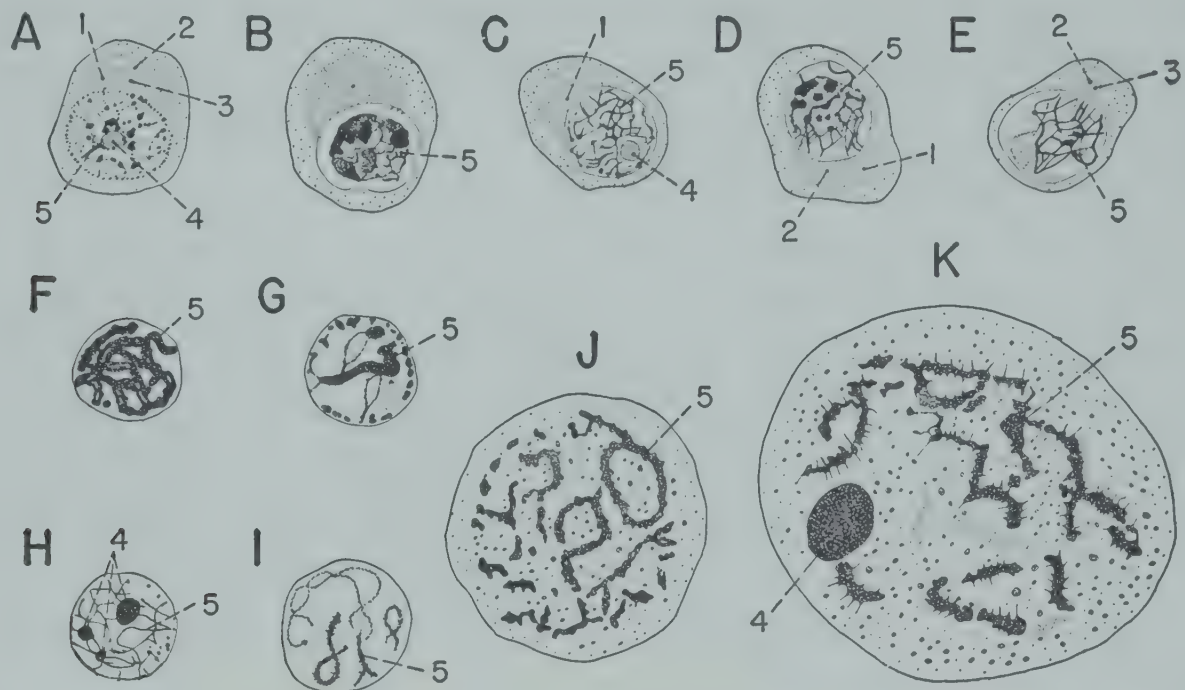
### Oögonia

The female germ cells may be called oögonia during their period of rapid division. As Swift (1915) pointed out, the oögonia are somewhat smaller than the primordial germ cells, but otherwise resemble them except for the arrangement of the mitochondria. These are now grouped around the attraction sphere and form the "mitochondrial cloud."

Hollander (1904) gave a detailed description of the complex composed of the attraction sphere and the mitochondrial cloud, but designated by him as the "yolk-body of Balbiani." He spoke of the density of the outer zone (consisting of mitochondria) and noted that there is a clear, narrow intermediate zone surrounding the innermost portion with its central body



(Fig. 5-A and B). The entire structure fits over the nucleus like a cap. During the prophase of cell division, the mitochondria extend themselves around the nucleus, and, at anaphase, when the cytoplasm divides, each daughter cell receives half of them. The centrosomes of the division figure are apparently furnished by the central body, or bodies, of the attraction sphere.



**Fig. 5.** Nuclear changes occurring in primary oocytes of the chicken (*Gallus gallus*) during the last week of incubation and the first 3 weeks after hatching. (Redrawn with modifications after Hollander, 1904.)

A, cell with resting (reticulated) nucleus, from a 14-day embryo; B, cell containing nucleus with retracted mass of chromatin, from a 15-day embryo; C, cell from a 17-day embryo, with nucleus containing a fine filamentous network of chromatin; D, cell from a 17-day embryo, in synapsis; E, cell from a 19-day embryo, with nucleus in which the chromatin is unravelling from the synaptic stage; F, nucleus from oocyte of a 3-day-old chick, with chromatin in a single heavy filament split longitudinally in places; G, nucleus of oocyte from a newly hatched chick, with incompletely double filament of chromatin; H, nucleus from oocyte of a 6-day-old chick, in which the nucleolus has reappeared and the chromatin is arranged in a continuous filament; I, nucleus from intrafollicular oocyte of a 20-day-old chick, with chromosomes in rings and chains of the diplotene stage; J, nucleus from intrafollicular oocyte of 20-day-old chick, with chromosomes of irregular size and shape starting to withdraw from the nuclear membrane; K, nucleus of oocyte from a 20-day-old chick, with "barbed" chromosomes and a large nucleolus. All  $\times 600$ .

1, mitochondrial cloud; 2, centrosphere; 3, centrosome; 4, nucleolus; 5, chromatin or chromosomes.

The nucleus now contains a large nucleolus in the center, formed of several irregular masses. Chromatin granules are studded along the inner face of the nuclear membrane and along the threads of a very fine trabecular network that extends throughout the nucleus.

### Oocytes

When the oögonia cease multiplying, they enter a growth phase characterized chiefly by the accumulation of yolk in the cytoplasm. This period,



although of variable length, is protracted by comparison with the period of multiplication. The germ cells are now known as oöcytes, or, more strictly, as primary oöcytes. At the end of the growth period, the primary oöcytes undergo the first maturation division and give rise to the secondary oöcytes, which pass through the second maturation division and produce the mature ova. The first division does not take place until a few hours before ovulation, when the yolk is already full-grown, and the second division follows very shortly thereafter, or as soon as a spermatozoon enters the egg.

### *Nuclear Changes*

From the thirteenth to the fifteenth day of the chick's incubation period, the oöcyte presents so peculiar an appearance that Firket (1914) was led to believe that degeneration was occurring. He noted that the staining reaction of the entire cell becomes very poor and that the nucleus resembles an empty vesicle, except for a few badly defined masses of chromatin near the nuclear membrane. Hollander (1904) described the oöcytes of the fourteenth day (Fig. 5-A) as more regular and fine in structure than the oögonia. He also observed that, in oöcytes of the fifteenth day (Fig. 5-B), the only chromatin material present in the nucleus is collected in a mass enclosing the nucleolus and separated from the nuclear membrane by a thin, clear border. Between the sixteenth and eighteenth days, both filaments and clumps of chromatin are present, and the nucleolus is found at the periphery of the nucleus (Fig. 5-C). The chromatin threads then become retracted and arrange themselves in a tangled mass (synapsis) which is more compact centrally (Fig. 5-D). By the eighteenth or nineteenth day, the threads form looser loops and then begin to unravel; the karyoplasm regains its normal staining reaction, and the nucleolus disappears (Fig. 5-E). Goldsmith (1928) considered the nuclear changes of this entire period as synizesis leading to the leptotene stage (the first stage of the prophase of meiosis), with the zygotene (second) stage appearing on the twentieth day. Because the early stages of synizesis may suggest degeneration, he regarded it as understandable for Firket to conclude, erroneously, that there is degeneration of the primordial germ cells and "neoformation" of oöcytes from the germinal epithelium.

Goldsmith (1928) further noted that the leptotene threads persist through the second day post-hatching (Fig. 8-A) and disappear by the tenth day, at which time the nucleus is in the resting stage. Hollander (1904), however, stated that the threads become much thicker on the nineteenth or twentieth day of incubation and appear to be split longitudinally to a marked degree about at the time of hatching, or shortly thereafter (Fig. 5-F and G). His remarks indicate, therefore, that the nucleus passes out of the leptotene stage, through the pachytene stage, in which the chromosomes are paired, and probably into the diplotene stage. Sluiter



(1940) observed a series of nuclei in all stages of prophase in a single embryo of 14 to 15 days.

The nucleus in the oöcyte of the 4-day-old hatched chick is about 6 microns in diameter, the entire cell measuring 10 to 20 microns in diameter (*Brambell, 1926*). Differences in the rate at which the oöcytes develop now become apparent. In some nuclei, the chromatin is in a pseudoreticular stage (Fig. 5-H), with as many as three conspicuous nucleoli. However, *Hollander (1904)* noted that, between the sixth and the twentieth day after hatching, the chromatin may also be found in the diplotene stage (Fig. 5-I), from which it passes through a granular phase and becomes retracted from the nuclear wall (Fig. 5-J), finally forming the barbed or "lamp-brush" chromosomes (Fig. 5-K) mentioned by *Holl (1890)*. According to *Goldsmith (1928)*, on the other hand, there is no nuclear activity until the chick is about 10 weeks old, when the nucleus is again seen in the pachytene stage. The chromatin is arranged in thick, heavy threads, the number of which is obviously haploid. The nucleus then returns once more to the resting stage and remains there until the final, rapid growth phase that begins shortly before ovulation. The length of this resting period is extremely variable, for several years may elapse before some oöcytes mature. It is not possible, therefore, to give exact time limits to any of the stages of ovular development described here.

*Loyez (1906)* and *Durme (1914)* resumed the history of the oöcyte in the mature bird. In the smaller germ cells—which are about 50 microns in diameter in the chicken (*Brambell, 1926*)—the nucleus occupies an eccentric position in the cell. The threads composing the chromatin network grow thinner and become striated transversely. Multiple granules appear along the loops (Fig. 6-A). The filament then breaks into segments, and its fragments become transformed into barbed chromosomes (Fig. 6-B). From now on, the relative amount of chromophile material in the nucleus becomes steadily reduced. The chromosomes are at first arranged roughly in a circle, but they grow continually shorter and finally disaggregate (Fig. 6-C). Their period of existence apparently varies in different species of birds; *Durme (1914)* noted that it is relatively short in the swallow (*Hirundo rustica*) and long in the pigeon (*Columba livia*). According to *Sonnenbrodt (1908)*, the disintegration and disappearance of the chromosomes proceed very slowly in the chicken (*Gallus gallus*).

Chromosomes do not reappear until the nucleus has attained a position close to the periphery of the greatly enlarged oöcyte (*Sonnenbrodt, 1908*) at a considerably later time. They do not possess barbs, but are long, slender, double filaments (Fig. 6-D). Soon they form the arches, circles, diamonds, and other figures typical of the diplotene stage (Fig. 6-E). In cross section, the nucleus is no longer circular, but elliptical. Very soon, it is applied to the vitelline membrane, which surrounds the oöcyte, and as-



sumes a plano-convex shape (Fig. 6-F); in tangential section, however, it is still circular (Olsen, 1942).

The chromosomes now roll up into irregular masses which occupy a central position in the nucleus, among numerous small, scattered granules. Long, fine chromosomes are again reconstituted, and, as before, are formed of double filaments. These elements grow shorter and thicker and become concentrated in the middle of the external edge of the nucleus. In shape, they resemble figure eights, rings, diamonds, dumbbells, crosses, and other forms characteristic of diakinesis (the final stage of the prophase).

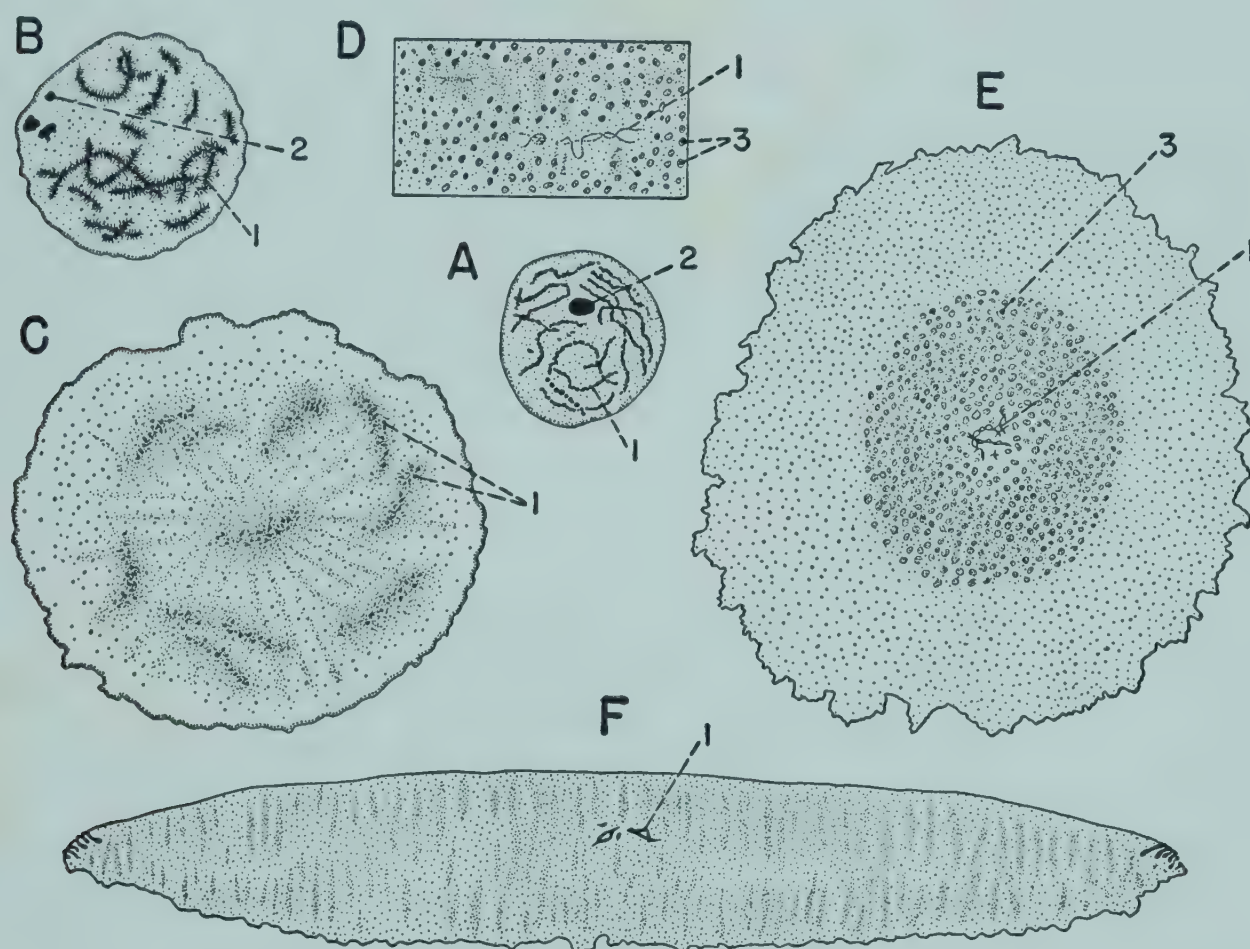


Fig. 6. Various forms assumed by the chromosomes in primary oocytes of the chicken (*Gallus gallus*) at successive stages of formation. (Redrawn with modifications from Sonnenbrodt, 1908.)

A, nucleus of an oocyte  $0.06 \times 0.08$  mm. in diameter, from a chick 14 days old; the chromosomes resemble streptococci and the nucleus contains one large nucleolus and many fine granules ( $\times 400$ ); B, nucleus of an oocyte  $0.09 \times 0.14$  mm. in diameter, from a chick 21 days old; the chromosomes are barbed and two of the three nucleoli are undergoing disintegration ( $\times 400$ ); C, nucleus of an oocyte  $0.75 \times 0.96$  mm. in diameter, from a chicken about 6 months old; the disaggregating chromosomes are represented by diffuse accumulations of granules ( $\times 200$ ); D, several looped, granular, lightly staining pairs of chromosomes found in the nucleus of an oocyte  $2.6 \times 2.9$  mm. in diameter, from an adult chicken; "chromatic nucleoli" are scattered throughout the nuclear fluid ( $\times 200$ ); E, nucleus of an oocyte  $5.8 \times 6.1$  mm. in diameter; a group of looped, deeply staining, paired chromosomes is seen in the center of a circular area filled with small "chromatic nucleoli" ( $\times 200$ ); F, elongated oval nucleus (sectioned vertically) from a 37-mm. oocyte. The short, thick, double chromosomes are in configurations characteristic of diakinesis ( $\times 200$ ).

1, chromosomes; 2, nucleolus; 3, "chromatic nucleoli."



The germinal vesicle begins to disintegrate about 24 hours before ovulation in the chicken, *Gallus gallus* (Olsen, 1942). At first, the only change is a slight wrinkling and folding of the nuclear membrane (Loyez, 1906); but Harper (1904) noted that the breakdown of the membrane is soon indicated in the oöcyte of the pigeon (*Columba livia*) by the appearance of a zone of clear nuclear substance around the germinal vesicle (Fig. 7-A). Some of the contents of the vesicle then spreads laterally to form a thin layer beneath the vitelline membrane, and some seems to move centripetally also (cf. Fig. 7-A). The first maturation division is now imminent.

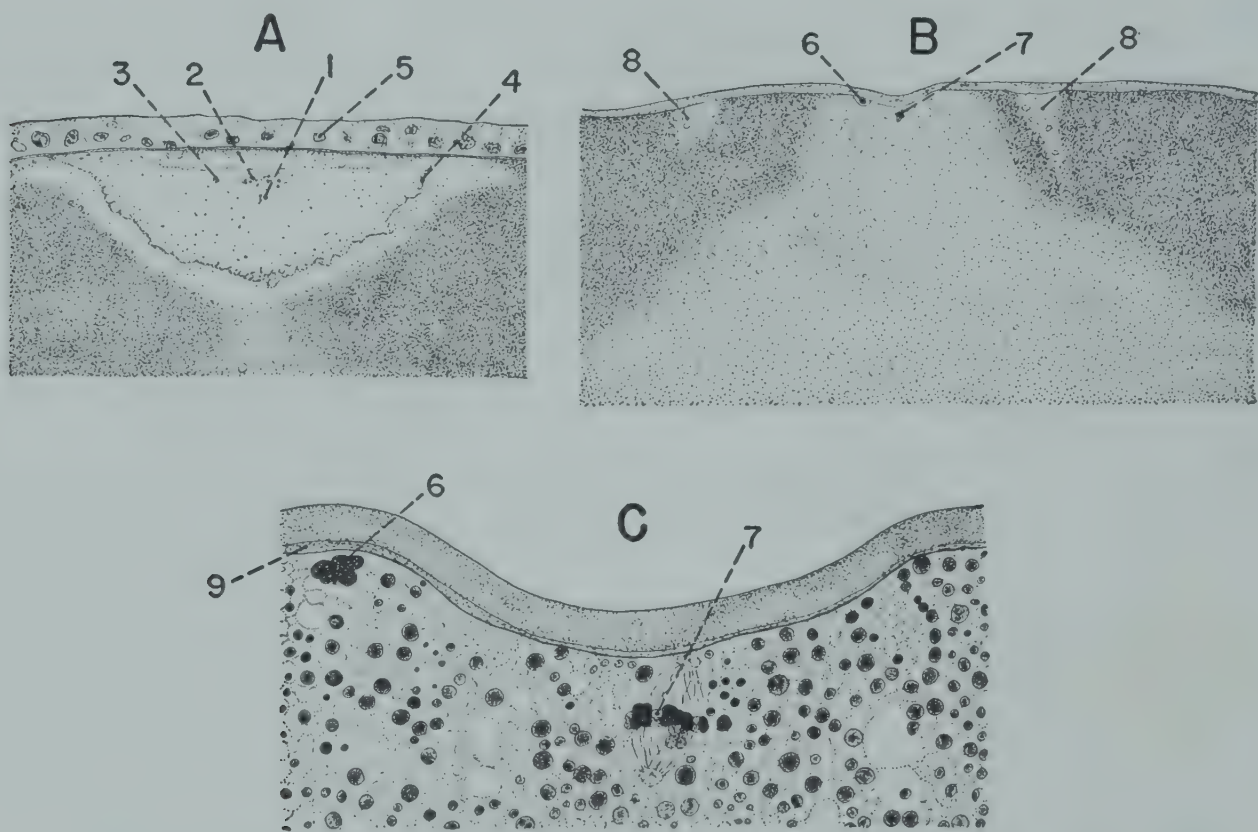


Fig. 7. Nuclear changes during late stages in the formation of the pigeon's (*Columba livia*) ovum. (Redrawn with modifications after Harper, 1904.)

A, nucleus of oöcyte about 2.0 cm. in diameter. The disintegrating nuclear membrane is wrinkled and surrounded by a clear zone of nuclear ground substance ( $\times 160$ ); B, vertical section through the germinal disc of a pigeon's egg after the disintegration of the germinal vesicle and the formation of the first polar body and the second polar spindle ( $\times 120$ ); C, an enlarged view of the polar body and spindle shown in B ( $\times 1200$ ).

1, group of chromosomes; 2, group of nucleoli; 3, refractive substance; 4, nuclear membrane; 5, nucleus of follicular cell; 6, first polar body; 7, second polar spindle; 8, "polar ring"; 9, perivitelline space.

**The First Maturation Division.** Through the maturation divisions, the diploid number of chromosomes is reduced to the haploid number, and the egg is thus prepared for fertilization. There are two reduction divisions, so-called, but the number of chromosomes is supposedly reduced during the first division. Each reduction division results in the formation of a polar body, composed almost entirely of chromatin and destined to degenerate. Theoretically, the first polar body should undergo a division that produces



an additional body, but this division fails to occur in many animals and has never been seen in birds.

The meiotic spindle presaging the formation of the first polar body has been seen in yolks removed from the pigeon, *Columba livia* (Harper, 1904) and the hen shortly before the estimated time of ovulation. The chromosomes are described as lying in an equatorial band the axis of which is slightly oblique to the surface of the yolk. In the hen (*Gallus gallus*), the first maturation division begins 4 or 5 hours before ovulation (Olsen and Fraps, 1950).

In this meiotic spindle, only the haploid number of chromosomes can be counted. Each chromosome is bivalent, consisting of a pair of identical elements. In cross section, the bivalent chromosomes appear as quadripartite bodies, or tetrads, because of the incipient longitudinal splitting of each member of the pair. The paired elements separate during the first meiotic division, but the longitudinal splitting is not completed until the second meiotic division, which is of the equational type.

The first polar body has been seen in unovulated yolks of the pigeon, *Columba livia* (Harper, 1904; Durme, 1914), and the swallow, *Hirundo rustica* (Durme, 1914), and also in yolks removed from the hen, *Gallus gallus* (Olsen, 1942; Olsen and Fraps, 1950), and the turkey, *Meleagris gallopavo* (Olsen and Fraps, 1944). It is a minute, oval globule lying just beneath the vitelline membrane and composed of chromatin and a small amount of karyoplasm (Fig. 7-B and C). According to Harper (1904), the first polar body retains its kinetic tendency, for its chromatin remains massed and shows no indication of forming a network. In the chicken, the first polar body is extruded not later than 2.5 hours prior to ovulation (Olsen and Fraps, 1950).

Almost immediately after the appearance of the first polar body, the second maturation spindle (cf. Fig. 7-B and C) begins to form (Harper, 1904; Durme, 1914; Olsen, 1942). However, meiosis is arrested in metaphase until the ovum has been ovulated and the spermatozoon has entered the egg (Olsen and Fraps, 1950).

### *Cytoplasmic Changes and Yolk Formation*

The growth of the oöcyte, due almost entirely to the formation of yolk and its deposition in the cytoplasm of the cell, proceeds neither continuously nor at a uniform rate. In general, it is very slow, except for the last few days before ovulation, when it is relatively very rapid. Both the slow and the rapid period are correlated with certain phases of yolk formation (Marza and Marza, 1935).

The period of slow growth occurs in two stages corresponding to an early and an intermediate phase in yolk formation. Beginning immediately after the period of cell multiplication, the first stage lasts until the oöcyte attains



a diameter, in the chicken, of about 2 mm. During this stage, yolk formation is characterized principally by the deposition of fat in the cytoplasm of the oöcyte (*Durme, 1914*). The second stage of the slow growth period begins when the diameter of the (chicken) oöcyte is about 3 mm. and continues until it is 6 mm. In oöcytes of this range in size, clear vacuoles appear in the cytoplasm and yolk is deposited within the vacuoles (*Durme, 1914*). During the period of rapid growth, the rate of increase in size is twenty-five times as great as previously (*Marza and Marza, 1935*), and the cell achieves a diameter of about 35 mm. (*Stieve, 1918*) through the deposition first of white yolk and then of layers of yellow yolk (*Durme, 1914*).

**The Early Phase of Yolk Formation.** The first phase of yolk formation begins before the end of the incubation period (about a week before, in the chick) and lasts for several months to several years. The varying length of this phase is due to the fact that it may be discontinuous. Growth and yolk formation may stop as soon as the oöcyte reaches 60 to 80 microns in diameter (*Hollander, 1904; Sonnenbrodt, 1908; Brambell, 1926*) and are not resumed until a few weeks or months before ovulation.

Age of Chick (days)	Diameter of Oöcytes (microns)
4	10– 20
21	34– 70
42	38– 75
77	44–100
Adult	50±

The figures given by Brambell (1926) indicate the growth rate of oöcytes in the chicken during the first few weeks after hatching. His data also show that the immature ovary contains some oöcytes which are larger than the smallest oöcytes in the adult ovary; they will presumably be the first to mature. Brambell also found oöcytes up to 380 microns in diameter in chicks 42 and 77 days old. However, atresia was apparent in cells exceeding 75 microns in diameter at 42 days and 100 microns in diameter at 77 days. He therefore concluded that many oöcytes grow rapidly in the period between 3 and 6 weeks after the hatching date but never reach maturity.

Early in this first period of yolk formation, the oöcyte becomes enclosed within a thin, intimately applied sac known as the follicle. About 2 days after the chick has hatched (*Goldsmith, 1928*), cells derived from the germinal epithelium can be seen aligning themselves around the oöcyte (Fig. 8-A). By the fourth to sixth day after hatching (*Hollander, 1904*) the oöcyte is surrounded by a ring of cuboidal cells constituting the early follicle (Fig. 8-B). Later, the cells of the follicular epithelium increase in height until they become pseudostratified; and then, during the final stages of yolk formation, they are stretched and flattened (*Marza and Marza,*



1935). The follicle is largely nutritive in function. It ruptures to permit the escape, or ovulation, of the fully formed egg.

The process of yolk accumulation in the intrafollicular oöcyte has been examined with great thoroughness by a number of workers, most of whom used material taken from the adult ovary. These observers are almost universally in agreement with Gegenbaur (1861), Waldeyer (1870), Holl (1890), and other early investigators, in designating two foci of yolk formation, one peripheral and one central.

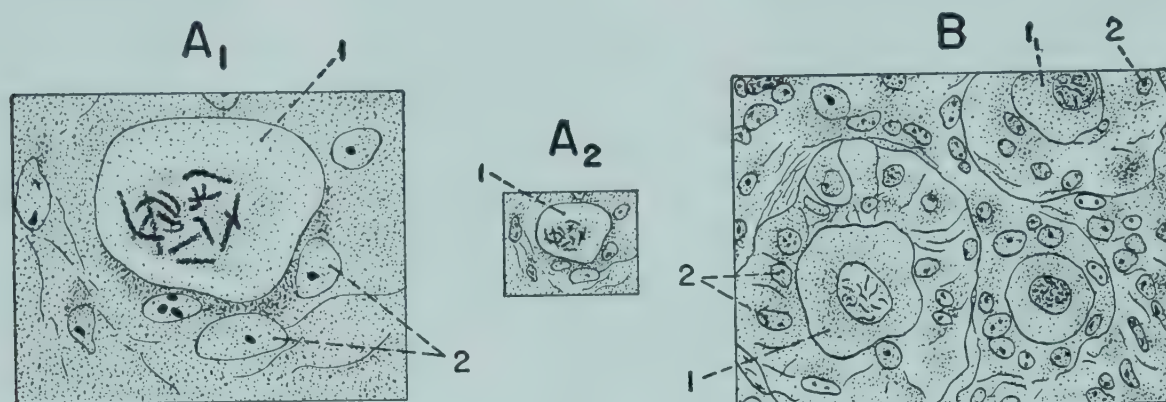


Fig. 8. Early stages in the formation of the follicle around the chicken (*Gallus gallus*) oöcyte. (Redrawn with modifications after Goldsmith, 1928.)

A<sub>1</sub>, section from the ovary of a 2-day-old chick, showing nuclei beginning to form a ring around an oöcyte ( $\times 1200$ ); A<sub>2</sub>, the same, at lower magnification ( $\times 400$ ); B, section of ovary of a 10-day-old chick, in which there is a ring of follicular cells about each oöcyte ( $\times 400$ ).

1, oöcyte; 2, nucleus of cell of early follicle.

Before these foci become apparent, and while the oöcytes are still small, certain changes occur in the cytoplasm. Among the first of these changes is an increase in the size of the mitochondrial cloud. This is already larger than the nucleus in oöcytes 50 microns in diameter (Fig. 9-C<sub>2</sub>), but it continues to grow and reaches its maximum development in cells 100 to 140 microns in diameter. Brambell (1926) noted that a homogeneous area, the "mitochondrial yolk body," 50 to 70 microns in diameter, then appears in the midst of the mitochondria (Fig. 9-E), apparently as the result of the condensation and fusion of some of these elements. This body is distinct from the centrosphere (Fig. 9-C<sub>1</sub>), which is surrounded by the Golgi apparatus (Brambell, 1926). On one side, the mitochondrial yolk body is in contact with the nucleus, and elsewhere it is separated from the mitochondria by a narrow, light zone. By the time the oöcyte has attained a diameter of 170 microns (Durme, 1914) to 260 microns (Brambell, 1926), the mitochondrial yolk body has disappeared and the mitochondria have spread out into the cytoplasm. The mitochondria are most numerous near the periphery, where they form a cortical layer (Fig. 10-D).

Brambell (1926) also made a detailed study of the changes in the Golgi apparatus during this same period. In cells measuring less than 75 microns



in diameter, the Golgi bodies are applied to the nucleus and are surrounded by mitochondria (Fig. 9-A and B). In somewhat larger cells, the Golgi bodies are still close to the nucleus, but they have separated distinctly from the mitochondria (Fig. 9-C<sub>2</sub>) and usually occupy the angle between the latter and the nucleus (Fig. 9-D and E). Small oöcytes also contain golgiosomes of follicular origin, intruded from the follicle cells. These

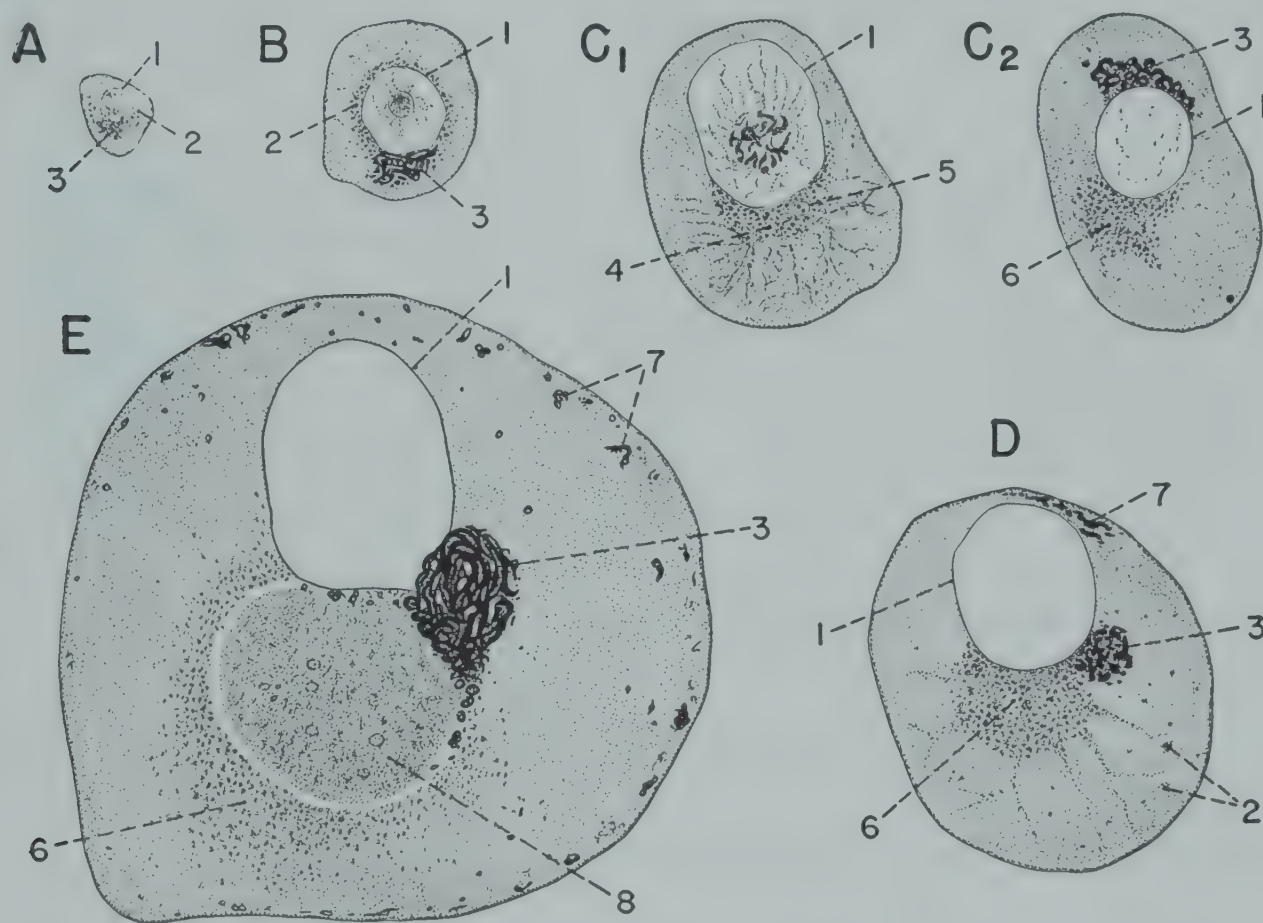


Fig. 9. The mitochondria and Golgi bodies in chicken (*Gallus gallus*) oöcytes 10 to 150 microns in diameter, demonstrated by cobalt-silver nitrate impregnation. (Redrawn with modifications after Brambell, 1926.)

A, an oöcyte about 12 microns in diameter, from a 4-day-old chick; B, an oöcyte about 30 microns in diameter, from a chick 3 weeks old; C<sub>1</sub>, an oöcyte, from a chick 6 weeks old, stained with iron-hematoxylin to reveal the centrosphere, the centrosome, and the two centrioles; C<sub>2</sub>, an oöcyte, from a chick 6 weeks old, containing the Golgi apparatus distinct from the newly formed mitochondrial cloud; D, an oöcyte about 75 microns in diameter, from a chick 11 weeks old, containing Golgi bodies intruded from the follicle; E, an oöcyte about 135 microns in diameter, from an adult fowl, containing Golgi bodies of intracellular and follicular origin, the mitochondrial cloud, and the mitochondrial yolk body surrounded by an outer clear zone. All  $\times 400$ .

1, nucleus; 2, mitochondria; 3, Golgi apparatus; 4, centrosphere; 5, centrosome; 6, mitochondrial cloud; 7, Golgi bodies intruded from follicle; 8, mitochondrial yolk-body.

appear first at the animal pole, close to the nucleus (Fig. 9-D), and then are found around the entire periphery of the cell (Fig. 9-E). As soon as the oöcyte reaches about 150 microns in diameter, the individual Golgi bodies of the Golgi apparatus proper break up, and, in all cells measuring more than 200 microns in diameter, the entire apparatus is dispersed throughout the cytoplasm as small granules. The Golgi bodies from the follicle cells,



on the other hand, become continually more numerous for some time, forming a layer at the periphery of the cell. In cells 800 microns in diameter, there is an enormous number of them, but they have disappeared from larger cells.

Fat spheres are seen in the general vicinity of the Golgi bodies of both types. In cells about 60 microns in diameter (Fig. 10-A), fat spheres sur-

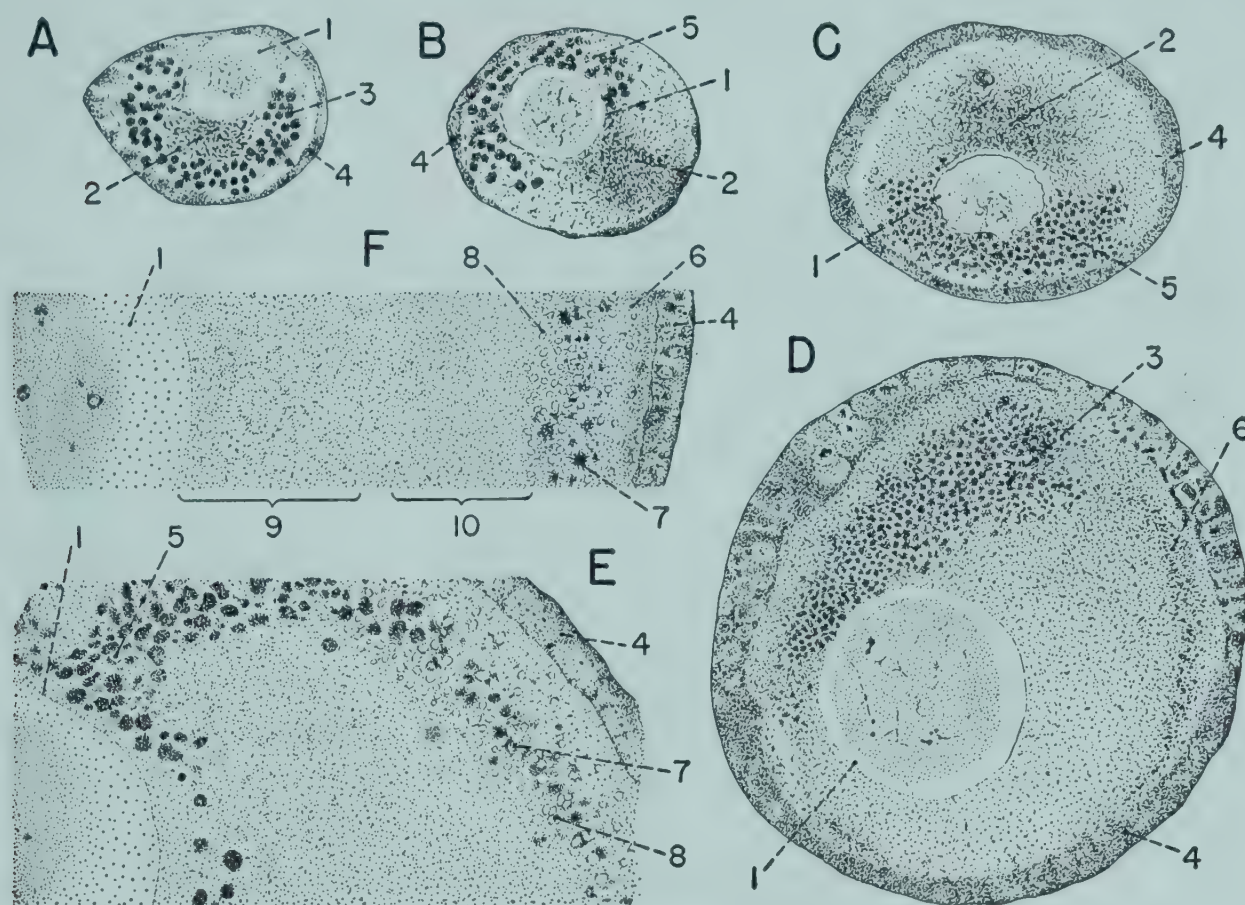


Fig. 10. Successive changes in the disposition of mitochondria and fat globules in maturing oocytes taken from a 5-month-old chicken (*Gallus gallus*) and an adult sparrow (*Passer domesticus*). Iron-hematoxylin stain. (Redrawn with modifications from Durme, 1914.)

A, chicken oocyte about 60 microns in diameter; B, chicken oocyte about 75 microns in diameter; C, chicken oocyte about 95 microns in diameter; D, chicken oocyte about 170 microns in diameter; E, portion of sparrow oocyte in which the nuclear cap of fat globules is being replaced by clear vacuoles, the fat globules have spread out to form a peripheral layer, and a few clear vacuoles are appearing among the peripheral fat globules; F, portion of sparrow oocyte in which the endoplasmic and exoplasmic zones have differentiated and a vacuolated layer is forming close to the peripheral fatty layer. All  $\times 230$ .

1, nucleus; 2, mitochondrial cloud; 3, fat; 4, follicular epithelium; 5, granulofatty nuclear cap; 6, cortical mitochondrial zone; 7, peripheral fatty layer; 8, layer of liquid yolk vacuoles; 9, endoplasmic zone; 10, exoplasmic zone.

round the mitochondria and Golgi elements, but in cells 75 microns in diameter (Fig. 10-B) they may be situated on the opposite side of the nucleus from the mitochondrial cloud (cf. Fig. 9-C<sub>2</sub>). For a time, an aggregation of fat globules forms a cap over the nucleus, between the nucleus and the vitelline membrane, which surrounds the ovum (Fig. 10-C). Considerably later, this cap spreads out (Fig. 10-D) and forms a layer of fatty deutoplasm (Fig. 10-E) just within the peripheral layer of mitochondria



(Fig. 10-E and F). Some fat still remains in the central portion of the cell, close to the eccentric nucleus (cf. Fig. 10-E). According to Konopacka (1933), the fat spheres are composed at first of phosphatides. As the number of spheres increases, neutral fats appear in the center of each sphere and eventually replace the phosphatides completely.

Approximately at the time that the barbed chromosomes start to disaggregate, the two regions where yolk formation is most active can first be distinguished (Durme, 1914). One of these is the endoplasmic (central) zone, composed of a large-meshed network of mitochondrial derivation;

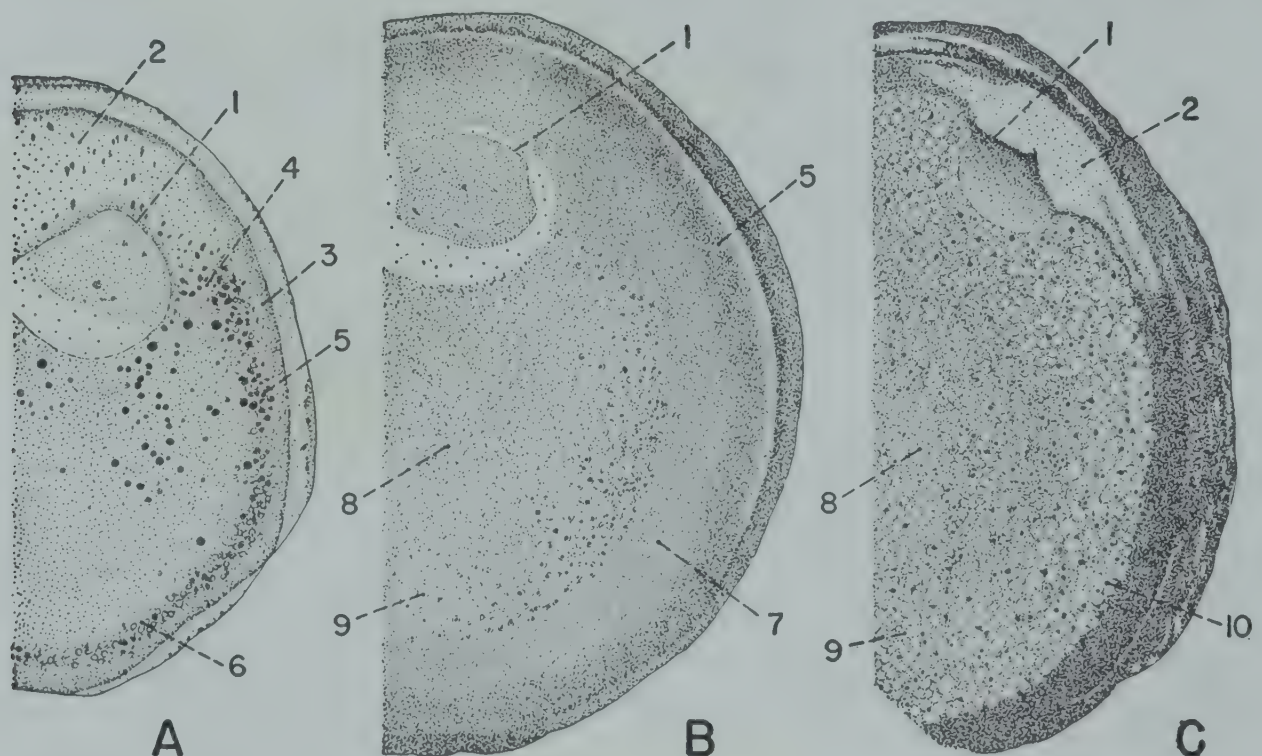


Fig. 11. Intermediate stages of yolk formation in the avian ovum. (Redrawn with modifications after Durme, 1914.)

A, oöcyte (about 260 microns in diameter) from an adult sparrow (*Passer domesticus*), at the stage in which protein vacuoles are beginning to appear within the peripheral fatty layer and the nuclear cap of fat globules has been replaced by one of clear vacuoles ( $\times 180$ ); B, oöcyte (about 975 microns in diameter) from an adult pigeon (*Columba livia*), at the stage in which a compact layer of very small yolk vacuoles is seen between the peripheral fatty layer and the exoplasmic zone of primordial yolk vacuoles containing large globules ( $\times 60$ ); C, oöcyte (about 875 microns in diameter) from an adult swallow (*Hirundo rustica*), at the stage in which oval globules of white yolk are forming peripheral to the exoplasmic zone ( $\times 60$ ).

1, nucleus; 2, vacuolar nuclear cap; 3, cortical mitochondrial zone; 4, fat globules; 5, peripheral fatty layer; 6, peripheral zone of vacuoles; 7, compact cortical layer; 8, endoplasmic zone; 9, exoplasmic zone; 10, white yolk layer.

the other is the exoplasmic (peripheral) zone, where the meshes of the network are smaller (cf. Fig. 10-F). The accumulation of yolk begins peripherally and proceeds toward the interior of the cell.

When the oöcyte (of the chicken) is about 1 mm. in diameter (Durme, 1914), clear vacuoles appear beneath the peripheral layer of fat spheres (Fig. 11-A; cf. Fig. 10-E and F). These vacuoles consist of clear, fluid protein (Loyez, 1906).



**The Intermediate Phase of Yolk Formation.** The oöcyte is still in the period of slow growth when it enters the second phase of yolk formation, or the vacuolar yolk phase, which lasts at least 60 days in the chicken (Marza and Marza, 1935).

In the 2-mm. oöcyte, clear yolk vacuoles, similar to those already present in the exoplasmic zone, appear in the endoplasmic zone (Durme, 1914). Descriptions of yolk formation during the period when the oöcyte grows from 2 to 3 mm. in diameter are meager. The follicle of the 3-mm. oöcyte becomes pediculated.

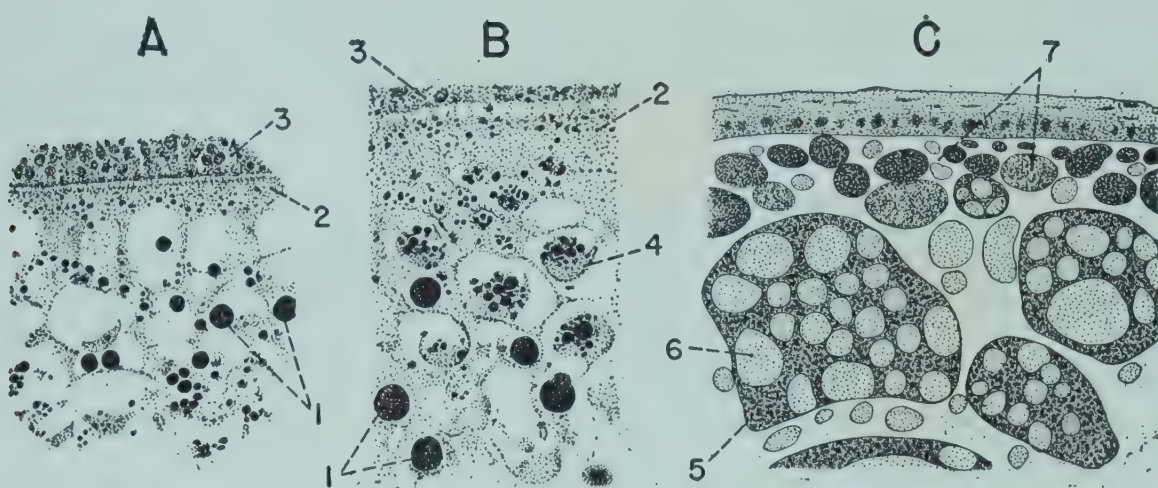


Fig. 12. Microscopic appearance of the various types of yolk successively deposited in the chicken (*Gallus gallus*) ovum during its formation. (Redrawn with modifications A and B, from Marza and Marza, 1935; C, from Konopacka, 1933.)

A, primordial yolk (vacuoles containing globules) in a 4.0-mm. oöcyte in the intermediate stage of yolk formation; B, the first true white yolk (vacuoles containing numerous small spherules) and primordial yolk in a 6.5-mm. oöcyte at the end of the intermediate phase of yolk formation; C, yellow yolk globules in an 8.0-mm. oöcyte in the final phase of yolk formation. All  $\times 130$ .

1, globule of intravacuolar primordial yolk; 2, granular cortical layer; 3, follicular epithelium; 4, white yolk globule with spherical intra-globular inclusions; 5, yellow yolk globule containing phosphoprotein granules; 6, fat droplet in yellow yolk globule; 7, layer of white yolk.

Small, round globules appear within the clear vacuoles of yolk when the oöcyte attains a diameter of 3 mm. (Fig. 11-B; Fig. 12-A). These globules gradually increase in size. Bissonnette and Zujko (1936) noted that they are deposited centripetally in the starling's (*Sturnus vulgaris*) oöcyte and that layers of small and large globules alternate.

According to Durme (1914) and Brambell (1926), the globules represent transformed mitochondria. Marza and Marza (1935), however, were of the opinion that mitochondria merely catalyze chemical changes in the oöplasm. Furthermore, Konopacka (1933) stated that the globules originate in the follicle as small granules which penetrate into the oöcyte and then into the vacuoles, and also indicated that they are composed of protein. The protein is probably the globulin, vitellin (Marza, 1935), combined



with phosphatides. Marza, Marza, and Chiosa (1932) stated that it is the constituent polypeptides of the vitellin molecule which appear first. This vacuolar type of yolk, termed "primordial yolk" by Marza and Marza (1935), is richer in water and protein and poorer in ash and fat than the yolk that will be formed later. Primordial yolk consists of 85 to 87 per cent water (*Romanoff, 1931; Spohn and Riddle, 1916*). Its solids contain 44 per cent protein, 11 per cent phosphatides (as lecithin), 23 per cent neutral fat, and 6 per cent ash (*Spohn and Riddle, 1916*), in which a small amount of organic iron is present (*Marza, Marza, and Chiosa, 1932*).

In the meantime, the fat spheres between the nucleus and the periphery of the cell have been replaced by a small mass of large, clear vacuoles capping the nucleus (Fig. 11-A). As these vacuoles accumulate, they grow out laterally (Fig. 11-C).

The peripheral and perinuclear vacuolated zones extend themselves rapidly. Soon the entire vitelline mass is vacuolated, the larger vacuoles always occupying the peripheral region.

**The Final Phase of Yolk Formation.** The final phase of yolk formation, coinciding with the period of rapid growth, begins (in the chicken) 6 to 14 days before ovulation occurs. For 1 to 5 days, white yolk is formed (*Marza and Marza, 1935*), and the diameter of the oöcyte increases from about 6 mm. to 8 to 10 mm. The growth rate accelerates sharply when the deposition of yellow yolk begins 5 to 9 days before ovulation (*Marza and Marza, 1935*). During this brief final stage of yellow yolk formation, the oöcyte becomes eighty times heavier (*Romanoff, 1931*) and attains a diameter of 35 mm.

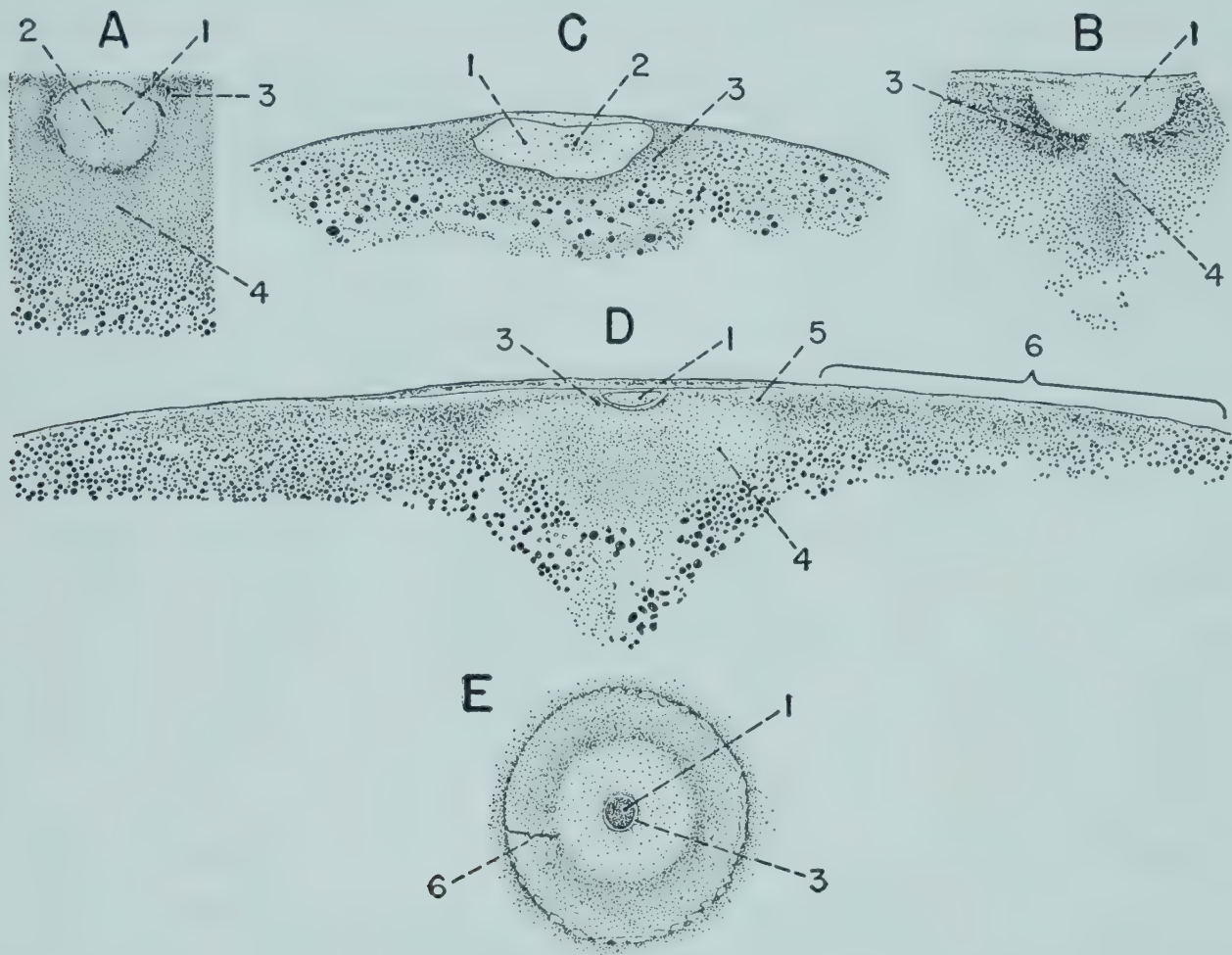
The oöcyte enters the final phase when a new peripheral zone of large yolk globules (cf. Fig. 11-C) differentiates just beneath the cortical layer of mitochondrial origin (*Durme, 1914*). This zone of large globules replaces a transitory compact layer of very small yolk globules (cf. Fig. 11-B). The large globules are not round, but irregularly oval, with many small granular inclusions at one pole (Fig. 12-B). These inclusions apparently result from the fragmentation of the phosphoprotein globules (*Konopacka, 1933*). The yolk of this layer was considered by Marza and Marza (1935) to be the first true white yolk. It contains more fat, including cholesterol, than primordial yolk, and less protein and water.

After the first white yolk layer appears, the peripheral fatty layer is no longer present (cf. Fig. 11-B and C), and the cortical layer of mitochondrial origin gradually becomes thinner and finally disappears. The nucleus, or germinal vesicle, continues to approach the periphery of the cell, compressing the nuclear cap and forcing it to spread laterally.

A new type of yolk composed of very fine granules—the "polar granules" of Bartelmez (1912)—now appears around the nucleus. This yolk is probably derived from the nuclear cap, or at least is formed under its influence



(Durme, 1914). In cross sections, this perinuclear yolk, or "plastic yolk" (Durme's term), in which segmentation will occur, is first seen extending to each side of the nucleus as a triangular mass (Fig. 13-A). Beneath the germinal vesicle, there is a round aggregation of very finely granular white yolk which is continuous with the almost central endoplasmic zone. This round mass is the primordium of the nucleus of Pander.



**Fig. 13.** Stages in the organization of the germinal region of the avian ovum. (Redrawn with modifications A and B, from Durme, 1914; C, D, and E, after Bartelmez, 1912.)

A, section through the germinal vesicle and adjacent regions of a swallow's (*Hirundo rustica*) oöcyte, in which the perinuclear "plastic yolk" has differentiated and the primordial nucleus of Pander can be seen immediately under the germinal vesicle ( $\times 40$ ); B, the same region of a chicken's (*Gallus gallus*) oöcyte at a somewhat later stage, when the perinuclear yolk is extending beneath the germinal vesicle ( $\times 40$ ); C, a still later stage in a pigeon's (*Columba livia*) oöcyte, in which the perinuclear yolk completely surrounds the germinal vesicle ( $\times 40$ ); D, the germinal region of a pigeon's oöcyte about 26 hours before ovulation ( $\times 40$ ); E, surface view of the blastodisc of a pigeon's oöcyte about 45 hours before ovulation ( $\times 7$ ).

1, germinal vesicle; 2, chromatin; 3, perinuclear "plastic" yolk (or "polar granules"); 4, nucleus of Pander; 5, "wedge" granules; 6, periblastic region.

After the chicken oöcyte has attained a diameter of 8 or 9 mm., its growth is due chiefly to the addition of large quantities of yellow yolk laid down at the periphery of the cell. Alternating strata of dark and light yellow yolk are often seen and probably result from variations in the amounts of yellow pigments deposited.

Yellow yolk globules are apparently formed from white yolk globules



(*Konopacka, 1933*) through the penetration of numerous, minute granules of fat into the latter (Fig. 12-C). Marza and Marza (1935) distinguished a type of yolk that is transitional between the white and the yellow forms. Yellow yolk is much richer in fat than white yolk (*Marza and Marza, 1935*); it contains large amounts of cholesterol (*Marza and Marza, 1932*). Marza (1935) noted that globulins (especially vitellin) predominate over pseudoglobulins (livetins) in the proteins of yellow yolk. Protein comprises only 28 per cent of the solid substance of yellow yolk (*Spohn and Riddle, 1916*), in which there is 45 per cent water (*Romanoff, 1931*). Neutral fats make up about 50 per cent of the solids (*Spohn and Riddle, 1916; Romanoff, 1931*), phosphatides 21 per cent, and ash 0.5 per cent (*Spohn and Riddle, 1916*).

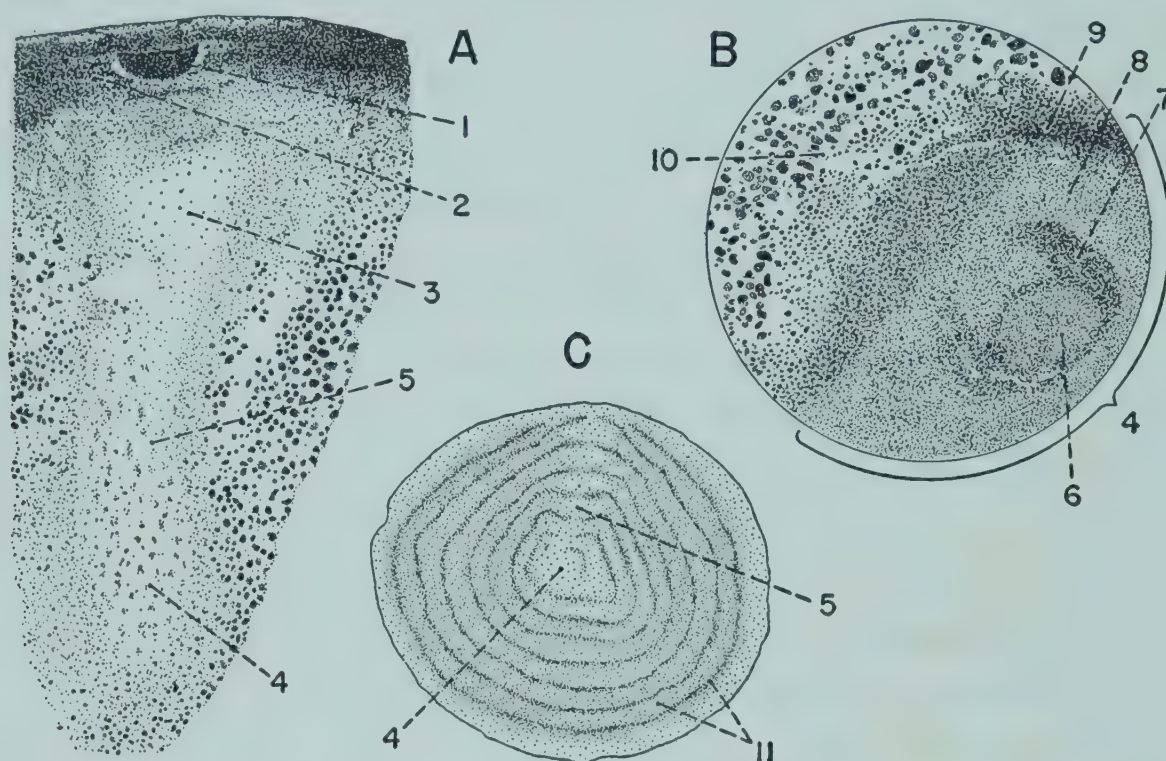
During this final period, the germinal vesicle and the white yolk beneath it are gradually separated as the perinuclear "plastic" yolk grows under the germinal vesicle from all sides (Fig. 13-B and C). The centrally located white yolk is now known as the latebra. In cross section, the latebra, which is about 6 mm. in diameter, is seen to be formed of concentric strata (Fig. 14-B). Marza and Marza (1935) believed that these strata correspond to the endoplasm, the exoplasm, and the first white yolk layer of the younger oöcyte, and pointed out that the similar size and chemical composition of the latebra and the younger oöcyte also indicate the identity of the two. Extending from the latebra toward the germinal vesicle is an elongated stalk of white yolk, the neck of the latebra (Fig. 14-A). This is possibly laid down in the wake of the migrating germinal vesicle (*Bartelmez, 1912*) or at least is formed under the influence of the latter. As Fig. 14-C shows, the layers of yellow yolk in the fully formed ovum are interrupted by the latebra and its neck.

Directly beneath the germinal vesicle there is an aggregation of white yolk in the form of an inverted cone. This is the nucleus of Pander, which is continuous centrally with the neck of the latebra. Immediately external or peripheral to the outermost edge of the nucleus of Pander there is a zone of perinuclear yolk which is wedge-shaped (*Bartelmez, 1912*) in cross section, tapering toward and merging with the mass of polar granules next to the germinal vesicle. The perinuclear yolk continues as a thin layer, the periblast, for some distance on the other side of the wedge-shaped region, that is, on the side farther removed from the germinal vesicle.

When viewed from the surface (Fig. 13-E), the germinal vesicle is seen to be surrounded by several concentric rings, which, proceeding from the center outward, are as follows: a very narrow, light, opaque polar ring; a wider, light ring above the nucleus of Pander; a dark, inner periblastic ring (of "wedge" granules) immediately peripheral to the nucleus of Pander; and the light, outer periblastic ring. These structures make up the entire germinal area.



By the time the nucleus undergoes maturation, the perinuclear yolk in the blastodisc has increased greatly in thickness and diameter, and the nucleus of Pander appears to have diminished in size. These changes are accentuated after the formation of the second maturation spindle (*Durme, 1914*). Harper (*1904*) observed that the first polar body and the second maturation spindle are located at the apex of a cone-shaped zone of clear substance derived, perhaps, from the liquid contents of the germinal vesicle.



**Fig. 14.** The latebra of the avian ovum. (Redrawn with modifications A, from *Durme, 1914*; B, from *Marza and Marza, 1935*; C, from *Warren and Conrad, 1939*.)

A, a section showing the latebra and its relationship to the nucleus of Pander and the germinal vesicle in an oviducal ovum of the sparrow, *Passer domesticus* ( $\times 30$ ); B, the concentric layers composing the latebra, as seen in a section of a 20-mm. chicken (*Gallus gallus*) oöcyte ( $\times 8$ ); C, section through the boiled yolk of an egg from a hen fed Sudan III, in which the latebra appears as a light central area and the location of the neck of the latebra is indicated by interruptions in the concentric dyed bands of yellow yolk (natural size).

1, germinal vesicle; 2, perinuclear "plastic" yolk ("polar granules"); 3, nucleus of Pander; 4, latebra; 5, neck of latebra; 6, central layer of latebra, formed of globules resembling those of the endoplasmic zone of small oöcytes; 7, layer formed of globules resembling those of the exoplasmic zone; 8, layer of globules resembling primordial yolk; 9, layer of white yolk; 10, yellow yolk; 11, dyed bands of alternating light and dark yellow yolk.

He found the polar ring still apparent at this stage (cf. Fig. 7-B). During the maturation period, perivitelline liquid appears between the follicle and the oöcyte (cf. Fig. 7-C).

A cortical layer of white yolk is found at the periphery of the fully formed oöcyte. In this cortical white yolk, vitellin and livetin are present in equal amounts (*Marza, 1935*), and the percentage of cholesterol, although higher than in yellow yolk, is lower than in the white yolk of the periblast



(Marza and Marza, 1932, 1935). Like the latebra, the cortical white yolk contains small amounts of nucleoproteins (Marza, 1935).

The outermost covering of the ovulated ovum is the vitelline membrane. According to McNally (1940, 1943), this is a fibrous, noncellular structure that constitutes the innermost layer of the follicle until the time of ovulation, when it separates from the follicle and adheres to the ovum. In the newly released yolk, the membrane is about 5 microns thick and is composed of collagen, although its thickness and composition both change before the egg is laid.

The albumen, shell membrane, and shell of the bird's egg are added as the yolk passes through the oviduct (Romanoff and Romanoff, 1949, pp. 174-252) and are the secretory products of this organ.

### The Bilaterality of the Ovum

As Bartelmez (1912) has pointed out, the bilateral organization of the egg and the position of the future embryo's long axis are determined while the ovum is still in the ovary and can be detected, in fact, early in the period of slow growth. In a small intrafollicular oöcyte, the nucleus occupies a peripheral position and thus marks one end of the polar axis. The long axis of the cell lies perpendicular to the polar axis and approximately parallel to the surface of the ovary. The polar axis does not bisect the long axis exactly, being slightly nearer one end of it than the other. The nucleus is very slightly elliptical, and its long axis is inclined to the long axis of the oöcyte.

As the oöcyte grows, it approaches the surface of the ovary, and the epithelium of its follicle comes into contact with the peritoneal epithelium of the ovary and fuses with it. The fusion necessarily occurs along the oöcyte's long axis, which, as noted above, parallels the ovarian surface. The area of fusion, known as the stigma, receives no blood supply. The bilateral arrangement of blood vessels tends to preserve the bilaterality of the oöcyte by regulation of yolk deposition.

Although the nucleus lies temporarily near the center of the cell, it is always slightly eccentric in the beginning and becomes increasingly so. Its eccentricity is responsible for the formation of the latebra and its neck closer to the animal (protoplasmic) than to the vegetal pole and nearer to one end of the long axis than the other. The white yolk of the central core is of lower specific gravity than the remainder of the oöcyte. The presence of this aggregation of white yolk forces the oöcyte to rotate within the follicle as soon as a space, the zona radiata, forms between the vitelline membrane and the follicular epithelium. Since the follicle hangs down in the body cavity by its pedicle, the animal pole of the oöcyte comes to rest directly beneath the pedicle when the rotation of the oöcyte brings it uppermost. Similarly, the eccentric placement of the latebra on the long



axis of the cell causes the heavier end of the oöcyte to gravitate toward the cloaca of the bird.

### Oögenesis in Hybrid Birds

The ovary in hybrids has been observed infrequently because, in birds, the offspring of intergeneric and interspecific crosses usually are preponderantly male (Guyer, 1909c; Smith and Haig Thomas, 1913; Haig Thomas and Huxley, 1927). The aberrant hybrid sex ratio may be due to differential embryonic mortality (Cole, Painter, and Zeimet, 1928a; Painter and Cole, 1943), sex reversal (Haig Thomas and Huxley, 1927; Riddle and Johnson, 1939), or causes not as yet elucidated. The deficiency of females is in accordance, however, with the observations of Haldane (1922), who called attention to the fact that the heterogametic sex ordinarily is greatly in the minority among hybrid animals.

In the few female hybrid birds that have been examined, the ovary is abnormal and may even be absent, as Phillips (1916) found it to be in hybrids between Reeves' pheasant (*Syrnaticus reevesii*) and the Prince of Wales pheasant (*Phasianus colchicus principalis*). Smith and Haig Thomas (1913), in studying female offspring of a Reeves' pheasant hen and a male Formosan pheasant (*Phasianus colchicus formosanus*), observed that the ovary was composed of nests of interstitial cells with stroma and fibrous tissue but without any germinal elements. These authors, speculating on the basis of their findings in the male hybrid, suggested that the oöcytes had degenerated as the result of incomplete synapsis during embryonic development, the incompatibility of maternal and paternal chromatin preventing the formation of bivalent chromosomes. Owen (1941a), reporting on the embryonic development of hybrids of the male chicken (*Gallus gallus*) and the guinea hen (*Numida meleagris*) indicated that the abnormal structure of the ovary is of very early origin. He remarked on the lack of activity shown by the germ cells but made no mention of abnormal synapses.

### SPERMATOGENESIS

Spermatogenesis resembles oögenesis in that it leads to an eventual halving of the number of chromosomes in the cell. The maturing of the sperm cell, however, is characterized not by growth but by a differentiation of form involving an actual loss of some of the protoplasm. Furthermore, the maturation divisions in spermatogenesis result in four cells capable of participating in fertilization, rather than one.

#### Spermatogonia

The primordial germ cells in the male chick (*Gallus gallus*) embryo begin to divide actively on or after the thirteenth day of incubation (Swift,



1916), a few days later than in the female. At the end of a 4-day period of multiplication, the mitochondria are grouped around the attraction sphere, and, according to Swift (1916), the cells may now be regarded as spermatogonia. They remain quiescent for some time, although their location changes as development of the testicle progresses. The process of male gonad formation need not be described here beyond mention of the fact that it leads to the eventual establishment of greatly convoluted seminiferous tubules in a stroma of connective tissue and interstitial cells. The

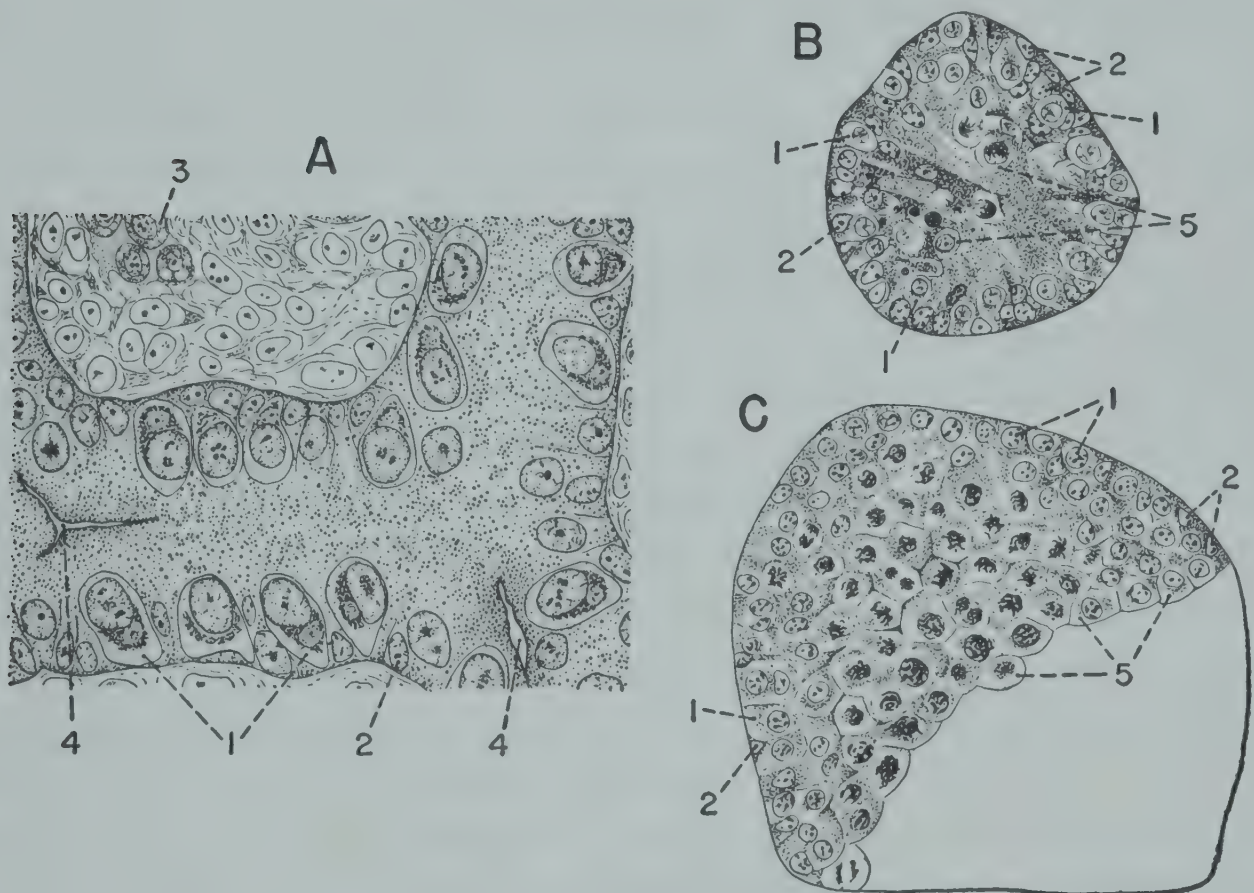


Fig. 15. Stages in avian spermatogenesis. (Redrawn with modifications A, after Swift, 1916; B and C, after Loisel, 1901.)

A, a section through part of a gonad of a 20-day chick (*Gallus gallus*) embryo ( $\times 1200$ ); B, a cross section of a seminiferous canal from the testis of an adult sparrow (*Passer domesticus*) in March ( $\times 250$ ); C, the same, somewhat later in March ( $\times 250$ ).

1, spermatogonium; 2, nucleus of germinal epithelial cell; 3, interstitial cell; 4, lumen developing in seminiferous cord; 5, primary spermatocyte.

walls of the tubules are formed of cells derived from the germinal epithelium, and the spermatogonia are interspersed among these cells. The tissue often resembles a syncytium, in which the spermatogonia stand out prominently. In the 20-day chick embryo, they line the basement membrane of the tubules and are oriented in such a way that their mitochondrial crescents are next to the basement membrane and their nuclei are turned toward the lumen, which is beginning to form in the hitherto solid cord through the liquefaction of the central cells (Fig. 15-A).



In the adult English sparrow (*Passer d. domesticus*)—a bird that has been used extensively for the study of spermatogenesis—the spermatogonia begin to divide only a few weeks before the first spermatozoa mature. The start of spermatogenetic activity is signaled by a migration of some of the spermatogonia toward the center of the tubule, where they may transform into spermatocytes (*Kirschbaum and Ringoen, 1936*). These precocious spermatocytes, however, seem destined for degeneration (*Loisel, 1901*). Within a few days, multiplication of the spermatogonia is in full progress, and soon great numbers of these cells are present. As their number is augmented, their size decreases, although the diameter of the nucleus does not change significantly (*Loisel, 1900*). Some of the spermatogonia remain near the periphery of the tubule and await a later date to take further part in spermatogenesis. Other cells are pushed to the interior, with the result that more than one layer of spermatogonia is formed (Fig. 15-B). By growth and nuclear change, the innermost spermatogonia become primary spermatocytes.

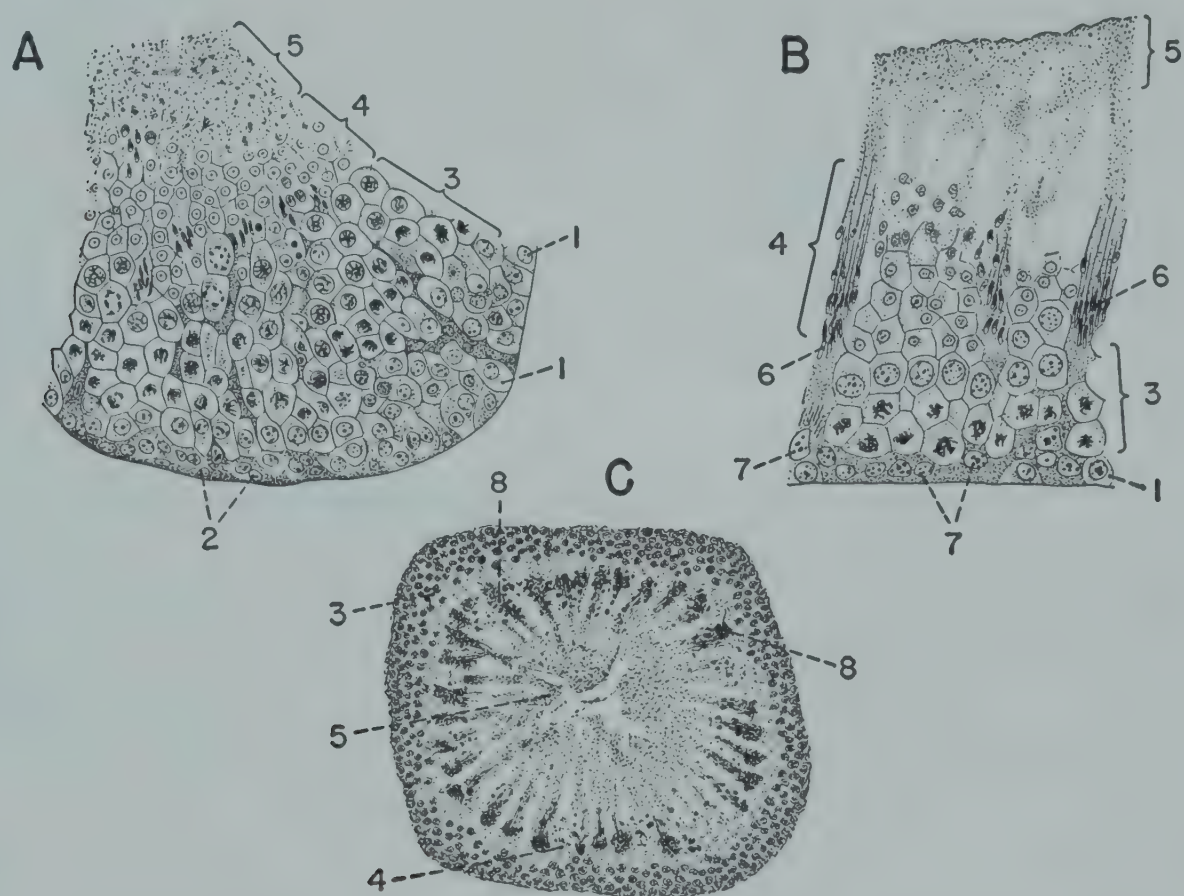
### Primary and Secondary Spermatocytes

The primary spermatocytes are much more numerous than the spermatogonia and eventually form a zone several layers thick, as shown in Fig. 15-C (*Loisel, 1901*). The youngest cells, located peripherally, are usually in either the resting, the leptotene, or the synaptene stage. The deeper layers are composed of older cells that progress through the pachytene, diffuse, and strepsitene (diplotene) stages to diakinesis, in which the chromatids are arranged as tetrads (*Guyer, 1916; Miller, 1938; Riley, 1938; Scaccini, 1942*). In the chicken, the mitochondria of the primary spermatocyte show signs of dispersal, but the Golgi material is arranged as a mass at one side of the nucleus (*Zlotnik, 1947*). By the time the cell is ready to divide, the Golgi material spreads around the nucleus in the form of a half moon, within which are two small bodies, either centrioles (*Miller, 1938*) or structures homologous to the accessory bodies of mammalian spermatocytes (*Zlotnik, 1947*). The centrioles have been observed to assume a V-shaped form in such diversified species as ducks, *Anas platyrhynchos* (*Korff, 1901; Baumgartner, 1931*), sparrows, *Passer domesticus* (*Riley, 1938*), and the guinea fowl, *Numida meleagris* (*Scaccini, 1942*).

In the sparrow, about a month is required for the nuclear changes leading to the first maturation division and the formation of the secondary spermatocytes, which contain the haploid number of chromosomes. These cells form a more internal layer than their predecessors (Fig. 16-A) and are relatively small, approximating the spermatogonia in size (*Guyer, 1916*). They are also much fewer in number than the primary spermatocytes, because, instead of accumulating, they divide almost immediately (*Loisel,*



1902; Riley, 1938). Observers of different species agree that the centrioles of the secondary spermatocytes are rod-shaped and that there is considerable clumping of the chromosomes (Baumgartner, 1931; Riley, 1938; Scaccini, 1942). In the chicken, both the Golgi bodies and the mitochondria are scattered in the cytoplasm during the first maturation division, but only the Golgi bodies reassemble in the secondary spermatocyte (Zlotnik, 1947).



**Fig. 16.** Late stages in spermatogenesis in the adult sparrow, *Passer domesticus*. (Redrawn with modifications A and B, after Loisel, 1901, 1902; C, from Kirschbaum and Ringoen, 1936.)

A, part of a section of a seminiferous tubule, in which may be seen all early stages of spermiogenesis ( $\times 200$ ); B, the same, but showing all stages of spermiogenesis ( $\times 200$ ); C, cross section of entire seminiferous tubule in same stage as B ( $\times 100$ ).

1, spermatogonium; 2, germinal epithelial cell; 3, zone of primary and secondary spermatocytes; 4, zone of spermatids; 5, zone of detritus in lumen of tubule; 6, spermatids in late stages of transformation into spermatozoa; 7, cell of germinal epithelium differentiating as Sertoli cell; 8, bundle of spermatozoa.

The division of the secondary spermatocytes (the second maturation division) yields spermatids. At one time, it was thought that a second conjugation, or pairing, of the chromosomes occurred prior to this division in the guinea fowl, *Numida meleagris* (Guyer, 1909a), the chicken, *Gallus gallus* (Guyer, 1909b, 1916), and the pigeon, *Columba livia* (Smith, 1912), so that the number of chromosomes appeared, superficially, to be halved again. Other observers have failed to corroborate this finding (Boring and Pearl, 1914; Hance, 1926b; Keith, 1934; Miller, 1938).



### Spermatids

The spermatids are small and have a clear peripheral zone surrounding a spherical nucleus in which there are a few granulations on a chromatin network (Loisel, 1902; Scaccini, 1942; Zlotnik, 1947) and along the nuclear membrane (Miller, 1938). A round, vacuolar structure, the acroblast or idiosome, replaces the centrosphere. In the guinea fowl, *Numida meleagris* (Scaccini, 1942) and the chicken, *Gallus gallus* (Miller, 1938), the idiosome is inconspicuous, but in the sparrow (*Passer domesticus*) it is prominent, capped by the Golgi apparatus (Riley, 1938). Miller (1938) stated that, in the chicken, the idiosome contains a central body; Zlotnik (1947) observed that the centrioles are located in the cytoplasm almost as soon as the spermatids form.

The spermatids metamorphose directly into spermatozoa without undergoing division. Although it has been indicated (Guyer, 1916) that half of the spermatids of the fowl divide anomalously, the daughter cells later degenerating, it is doubtful that this phenomenon occurs (Keith, 1934). In the sparrow (*Passer domesticus*), many untransformed spermatids appear to be carried away with the mature sperm cells (Loisel, 1902).

### Spermatozoa

The definitive sperm cell is formed merely through the rearrangement of the cellular material of the spermatid. The final product of this process of metamorphosis is a cell well adapted structurally for its function of propelling itself through the oviduct of the female and penetrating into the ovum.

#### *The Structure of Spermatozoa*

Avian spermatozoa, like those of other vertebrates, are elongated, flagellate cells, consisting of three principal parts: (1) the head, (2) the middle piece, and (3) the tail, in each of which there may be two or more regions or component structures. There is considerable species variation in the appearance of avian sperm cells (Fig. 17). Avian spermatozoa, however, are of two general types (Schweigger-Seidel, 1865), one characteristic of the passerine group and the other characteristic of all the remaining orders (Ballowitz, 1888). The latter is simpler and was regarded by Retzius (1909)

Length of Spermatozoa Heads	Frequency Distribution
(microns)	(per cent)
7.1– 8.0	0.4
8.1– 9.0	1.6
9.1–10.0	46.8
10.1–11.0	48.7
11.1–12.0	2.0
12.1–13.0	0.3
13.1–14.0	0.2



as the true sauropsid type. It is distinguished from the former chiefly by differences in the structure and relative size of the three main parts of the cell. However, the size of a part of the spermatozoon is not absolutely constant even within a species, as indicated by the tabulated data (Guyer, 1916) showing the varying length of the head in chick, *Gallus gallus* (Plymouth Rock), spermatozoa.

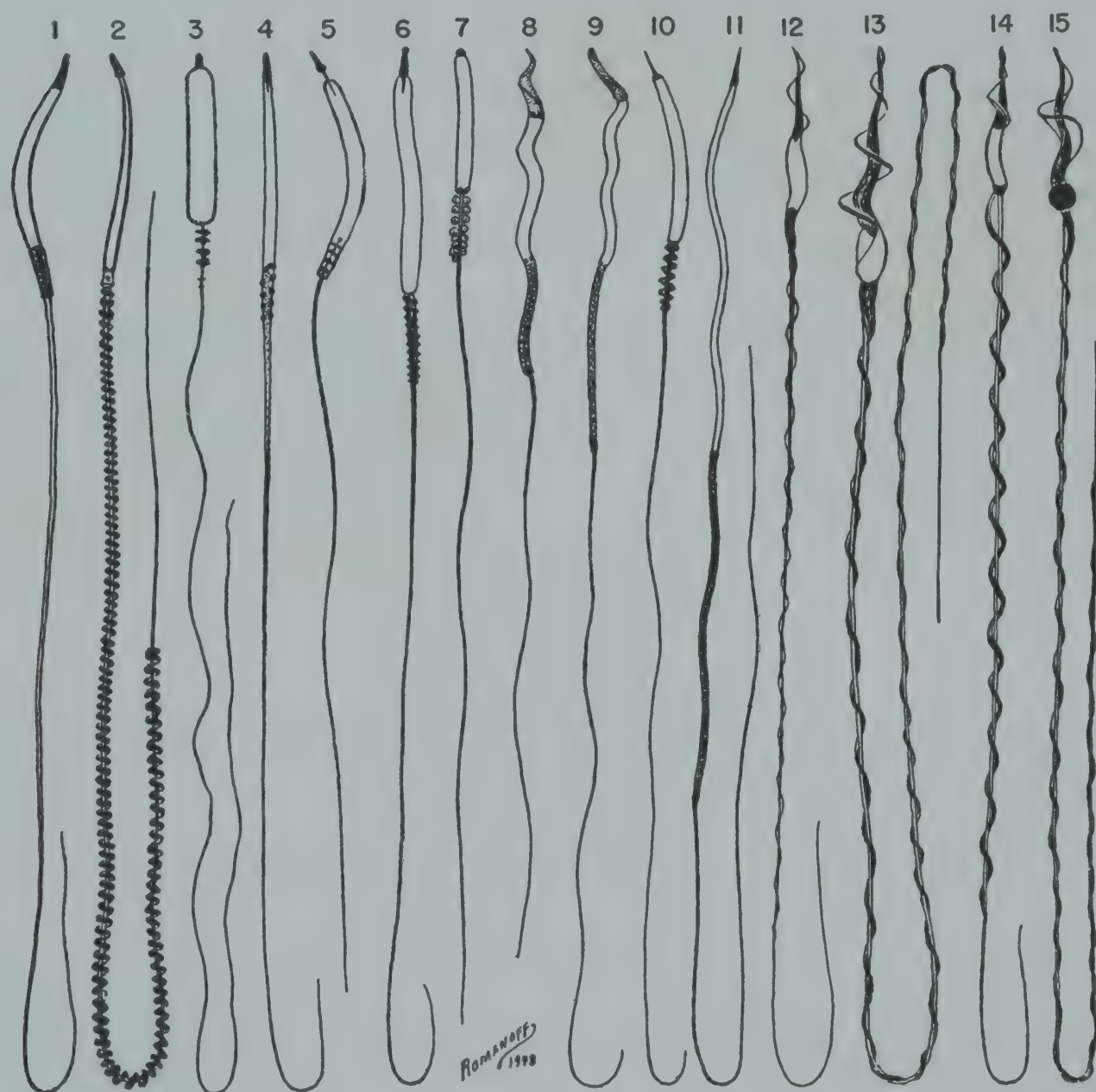


Fig. 17. Spermatozoa of various species of birds. (Redrawn with modifications 1, 2, and 4 to 15, after Retzius, 1909; 3, after Ballowitz, 1888.)

1, chicken (*Gallus gallus*); 2, pigeon (*Columba livia*); 3, turkey (*Meleagris gallopavo*); 4, duck (*Anas platyrhynchos*); 5, sea duck (*Aythya*); 6, ring-necked parrot (*Psittacus torquatus*); 7, black-backed gull (*Larus fuscus*); 8, red-backed sandpiper or dunlin (*Calidris alpina*); 9, European woodcock (*Scolopax rusticola*); 10, European coot (*Fulica atra*); 11, European ruff or sandpiper (*Philomachus pugnax*); 12, sparrow (*Passer domesticus*); 13, greenfinch (*Chloris chloris*); 14, song thrush (*Turdus philomelos*); 15, chaffinch (*Fringilla coelebs*). All  $\times 900$ .

In the less complex type of avian sperm cell, the head is long, narrow, straight or slightly curved, and cylindrical (Ballowitz, 1888). It is tipped by the pointed acrosome, which may contain an axial spine (Grigg and Hodge, 1949). The middle piece usually is fairly long—enormously so in some birds, such as the pigeon, *Columba livia* (Retzius, 1909). A slender spiral filament



may be twisted about the middle piece. The tail is shorter in proportion to the rest of the cell than it is in the passerine type.

The spermatozoa of passerine birds are distinguished by one outstanding feature, namely, their predominantly spiral configuration. The corkscrew turns are most conspicuous in the tail region but often traverse the entire length of the cell from anterior to posterior. The acrosome sometimes constitutes the larger part of the head. The middle piece is short, sometimes distinguishable with difficulty or not at all, and the tail is long.

### *The Formation of Spermatozoa*

Spermiogenesis, or spermateleosis—terms applied to the transformation of the spermatid into the spermatozoon—occurs in more or less the same way in all birds. The minor differences that have been reported may be actual or they may be the result of the varying techniques used by observers.

A detailed account of spermiogenesis in the sparrow (*Passer domesticus*), a typical passerine bird, has been given by Loisel (1902). The first change within the spermatid is the swelling of the acroblast to form a vesicle, apparently because of the diffusion of fluid into it from the nucleus (Fig. 18-A, B, and C). The Golgi bodies gradually move away from the vesicle (Riley, 1938); probably they are eventually excluded from the cell with a residuum of cytoplasm, as they are in the chicken (Zlotnik, 1947). The two centrioles then leave the acroblast, place themselves one behind the other, and proceed to migrate around the nucleus, one centriole remaining applied to the nuclear membrane during their passage (Fig. 18-C, D, and E). They eventually come to rest directly opposite the acroblast (Fig. 18-F). In the meantime, the cytoplasm has slowly liquefied near the periphery of the cell, although a denser perinuclear portion remains.

At the anterior end of the cell, the vesicular acroblast continues to grow. It becomes globular and depresses the anterior margin of the nucleus (Fig. 18-E, F, and G). One or two granules make a transitory appearance in the vesicle (cf. Fig. 18-F and G). Soon the acroblast elongates anterior to the nucleus, forming first a cone and then a cylinder, and finally takes three turns that transform it into the spiral acrosome (Fig. 18-H to Q, inclusive).

A somewhat different development of the acrosome was described by Brunn (1884) and Retzius (1909) as occurring in the sparrow (*Passer domesticus*) and also—according to the latter observer—in the European magpie (*Pica pica*), the hooded crow (*Corvus cornix*), the greenfinch (*Chloris chloris*), and other passerine birds. The acroblast differentiates into two regions, of which the anterior stains more lightly than the posterior. The latter portion gives rise to the acrosome proper and the former transforms itself into a spiral flange that borders the acrosome.

As the acrosome evolves, the nucleus undergoes changes that produce the head of the sperm cell. In the sparrow (*Passer domesticus*), the nucleus



loses its vesicular aspect and contracts, presumably because of the loss of fluid to the acroblast (Loisel, 1902). The chromatin becomes condensed into an amorphous mass (cf. Fig. 18-H), more acidophilic than the chromosomes and hence chemically different from them. This new substance uniformly invades the entire nucleus, in which no internal structure can be demonstrated by the more commonly used staining methods. The nucleus now elongates the curves slightly, continuing the spiral of the acrosome (cf. Fig. 18-I to Q, inclusive).

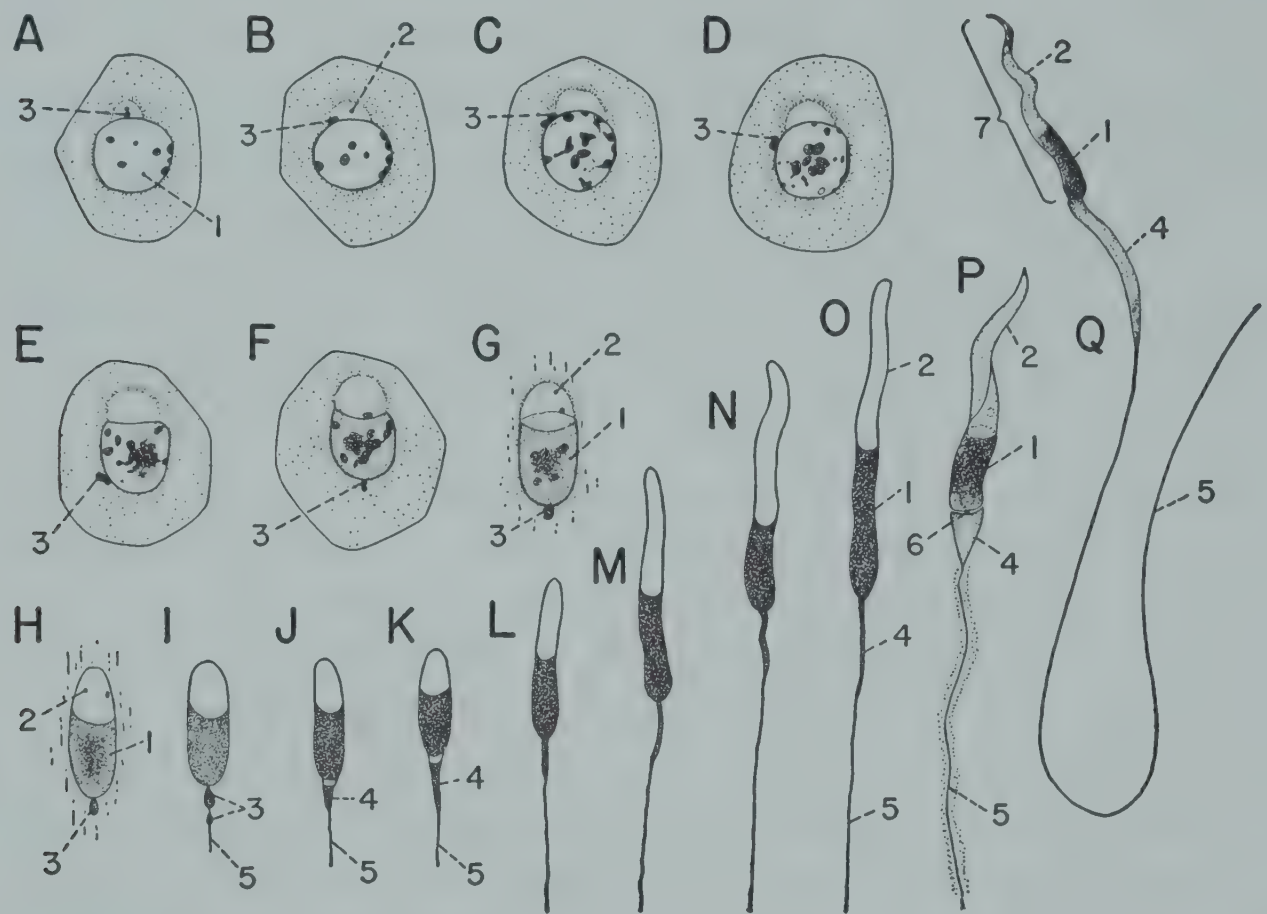


Fig. 18. Stages in the transformation of the spermatid into the spermatozoon, as observed in the sparrow, *Passer domesticus*. (Redrawn with modifications A to O, after Loisel, 1902; P and Q, after Riley, 1938.)

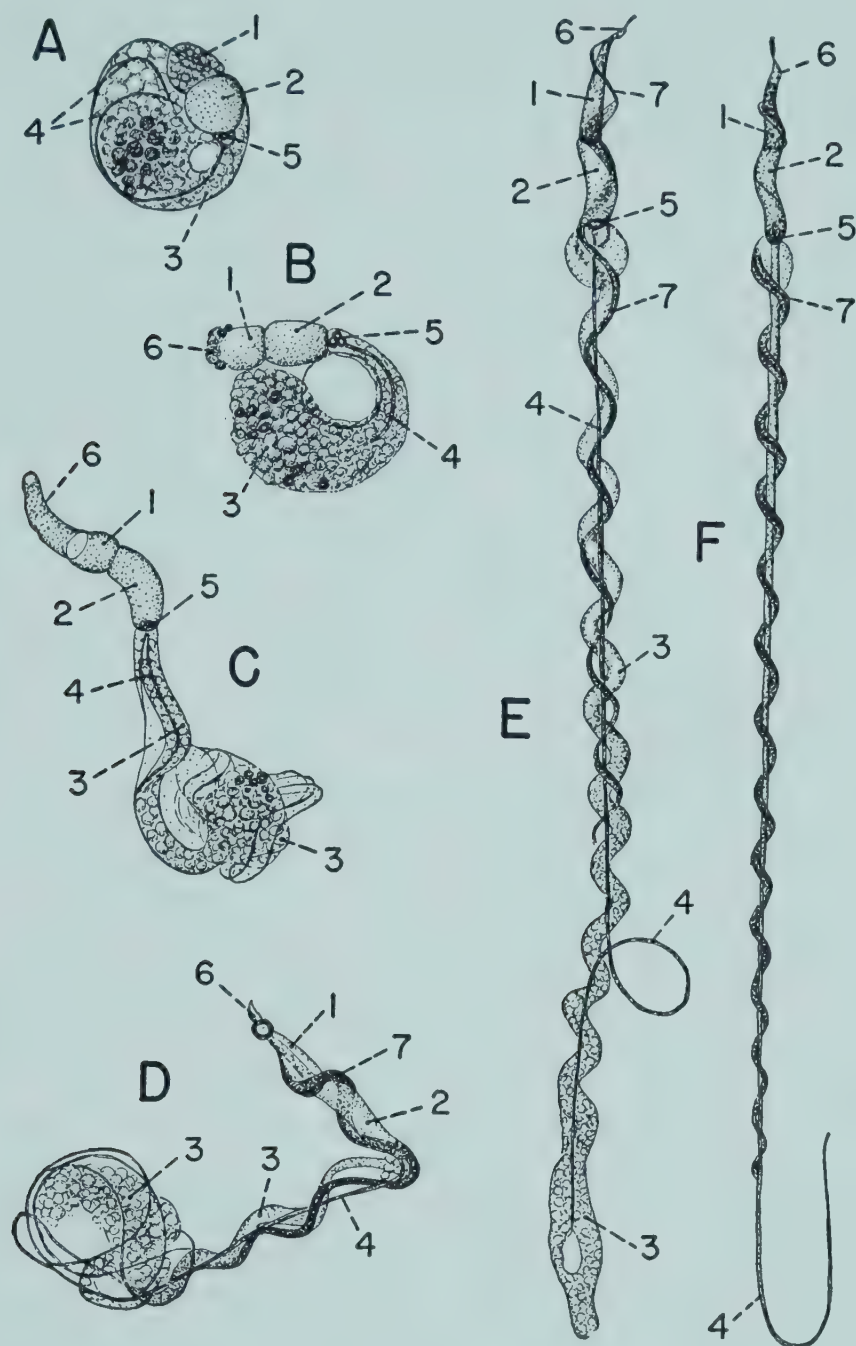
A to H, inclusive, show the migration of the centrosomes and the development of the acroblast into the acrosome; I to Q, inclusive, show the development of the middle piece and the axial filament of the tail, as well as the gradual changes in shape that produce the cell of definitive form. All  $\times 1500$ .

1, nucleus or nuclear portion of cell; 2, acroblast (archoplasmic vacuole) or acrosome; 3, centrosomes; 4, middle piece; 5, axial filament of tail; 6, caudal "manchette"; 7, head of spermatozoon.

The axial filament of the tail appears very early in spermiogenesis. According to Riley (1938), the axial filament in the sparrow (*Passer domesticus*) sperm cell takes its origin from the proximal centriole and passes through the distal one, which becomes ring-shaped. Loisel (1902), however, stated that the filament grows forth directly from the distal centriole (cf. Fig. 18-I). In some birds studied by Retzius (1909)—the song thrush (*Turdus philomelos*), the greenfinch (*Chloris chloris*), the chaffinch (*Fringilla coelebs*), and the European magpie (*Pica pica*)—the axial filament soon



attains great length and winds through the cytoplasm and over the surface of the round spermatid (Fig. 19-A and B). Retzius (1909) also noted a temporarily double axial filament in the developing magpie spermatozoon.



**Fig. 19.** The transformation of the cytoplasm of the spermatid during spermiogenesis in the song thrush, *Turdus philomelos*. (Redrawn with modifications after Retzius, 1909.)

A, B, the head and acrosome emerge from the cytoplasm, in which the axial filament can be seen; C, D, the axial filament extends and the cytoplasm begins to spiral around it. Two spiral structures, one light and one dark, are visible in D; E, a later stage; the double spiral is elongated and a clump of cytoplasm is visible around the end of the axial filament; F, a mature spermatozoon. The light and dark spiral structures have fused together. All  $\times 1100$ .

1, acrosome; 2, head; 3, cytoplasm; 4, axial filament; 5, centrioles; 6, cap of acrosome; 7, dark spiral filament.

In all avian species that he studied, Ballowitz (1888) found that the axial filament consists of very fine fibrils cemented together. This fibrillar structure is demonstrable only by certain laboratory procedures and is seen especially well under the electron microscope (Baylor, Nalbandov, and



Clark, 1943), which shows that nine fibrils compose the axial filament of the chicken sperm cell (Grigg and Hodge, 1949; Bonadonna, 1954). The fibrils are about 450 Å in diameter; an outer layer can be digested away with trypsin leaving an inner core (Grigg, 1951). Subfibrils can also be found under some conditions (Bonadonna, 1954). Grigg (1951) considers two of these to be primary structural elements located in the center of a ring formed by the larger fibrils.

In the sparrow (*Passer domesticus*), the proximal centriole soon prolongs itself and unites with the distal one to form a single mass, triangular in shape (cf. Fig. 18-I and J). This triangular mass gradually becomes cylindrical and elongated (cf. Fig. 18-K to Q, inclusive). These changes are apparently due to the deposition of a chromatic substance probably derived from mitochondria. Some of the same substance collects around the most anterior position of the axial filament, thereby enlarging its diameter (Loisel, 1902). The middle piece is thus formed around the centrioles and a short part of the axial filament. A similar origin has been ascribed to the middle piece in the European hooded crow, *Corvus cornix*, and the European magpie, *Pica pica* (Retzius, 1909).

The tail of the spermatozoon is derived from the cytoplasm of the spermatid. In passerine birds, the cytoplasm first passes to the caudal end of the spermatid, so that the nucleus and acroblast seem to be emerging from the round cell (cf. Fig. 19-A and B). As the cell elongates, the cytoplasm becomes drawn out along the axial filament (Brunn, 1884), retaining its globular shape only in its most posterior portion (Fig. 19-C, D, and E). In some birds, such as the magpie, *Pica pica* (Retzius, 1909), the caudal globule of cytoplasm contains a dark granule, which probably is cast out of the cell, eventually. The exclusion of considerable cellular material from the spermatozoon is indicated by the zone of detritus found next to the lumen of the seminiferous tubule (cf. Fig. 16-B and C). The continually lengthening mass of cytoplasm soon begins to assume a definite form, usually cylindrical, and to wind itself about the axial filament (cf. Fig. 19-E). The round terminal mass of cytoplasm finally disappears, and the spirally wound cylindrical structure tapers off somewhat anterior to the end of the axial filament, whose exposed tip is called the end piece (Fig. 19-F). In the song thrush (*Turdus philomelos*), the spiral filament of the tail is formed of two elements (cf. Fig. 19-E and F), at first separate but later fused (Retzius, 1909). The single spiral filament present in the spermatozoon of both the hooded crow (*Corvus cornix*) and the magpie (*Pica pica*) is sharply pointed at both ends, extends anteriorly to the region of the acrosome, and is apparently shed by the mature sperm cell in the ducts leading from the testis (Retzius, 1909).

In various nonpasserine birds, certain portions of the spermatozoon have been seen to evolve in a manner somewhat unlike that observed in pas-



serine birds. In the spermatid of the chicken (Guyer, 1909b; Keith, 1934; Miller, 1938), the domestic guinea fowl, *Numida meleagris* (Guyer, 1909a), and the domestic mallard duck, *Anas platyrhynchos* (Retzius, 1909), the chromatin has been observed to draw to one side of the nucleus and then to form a crescentic mass (Fig. 20-A to D, inclusive). This mass, tipped by the acrosome, grows toward the posterior end of the cell and is forced to coil up (Fig. 20-E, F, and F<sub>1</sub>) because the cytoplasm of the spermatid

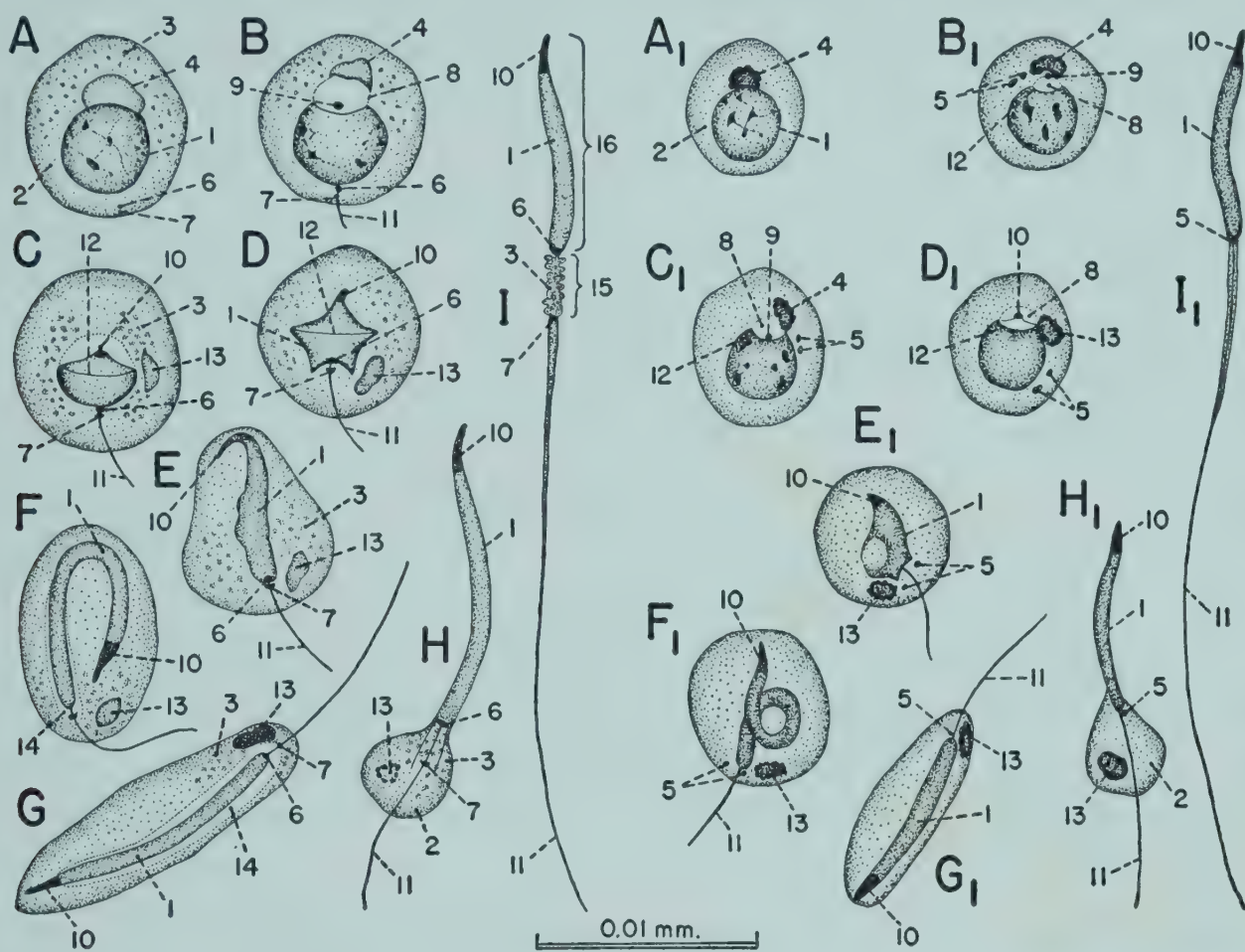


Fig. 20. Stages in the transformation of the spermatid into the spermatozoon in the chicken, *Gallus gallus*. (Redrawn with modifications after Zlotnik, 1947.)

A to I, cells stained to demonstrate the mitochondria; A<sub>1</sub> to I<sub>1</sub>, cells stained to demonstrate the Golgi apparatus. All in scale.

1, nucleus; 2, cytoplasm; 3, mitochondria; 4, Golgi material; 5, accessory Golgi body; 6, proximal centriole; 7, distal centriole; 8, archoplasmic vacuole; 9, proacrosome; 10, acrosome; 11, axial filament; 12, nuclear ring; 13, remnant of Golgi material; 14, clear zone or manchette; 15, middle piece; 16, head of spermatozoon.

retains its original shape (Zlotnik, 1947). The head of the spermatozoon eventually uncoils and emerges (Fig. 20-G, G<sub>1</sub>, H, and H<sub>1</sub>) from the cytoplasm (Guyer, 1909b, 1916; Keith, 1934; Zlotnik, 1947). In the mature spermatozoon of the guinea fowl (Guyer, 1909a) and of the chicken (Guyer, 1909b), the head contains beadlike refractile bodies whose fatty nature is indicated by their affinity for osmic acid (Adamstone and Card, 1934a). Digestion of chicken spermatozoa by nuclease also reveals that the head is not homogeneous but contains material, arranged as a fine spiral filament, which is not attacked by the enzyme and which therefore is not chromatin (Herwerden, 1916).



Zlotnik (1947), in describing the formation of the acrosome of the chicken spermatozoon, noted that the Golgi bodies are pushed away from the nucleus by the acroblast (Fig. 20-A<sub>1</sub> and B<sub>1</sub>). Directly under the Golgi material, at the anterior end of the acroblast, a small granule, the pro-acrosome, appears (Fig. 20-C<sub>1</sub>). This migrates across the acroblast to become the acrosome (Fig. 20-D<sub>1</sub>). A somewhat similar origin of the acrosome in the spermatozoa of some passerine birds has been described.

The ultimate fate of the Golgi material of the chicken spermatid was also observed by Zlotnik (1947). He stated that one accessory body originating within the Golgi apparatus is included in the neck region of the spermatozoon and that the remainder of the Golgi material is sloughed off with the residual cytoplasm (cf. Fig. 20-B<sub>1</sub> to H<sub>1</sub>, inclusive).

In the chicken spermatid, the mitochondria have been seen to migrate posteriorly in an orderly fashion (Zlotnik, 1947). Their deposition around the forward part of the axial filament differentiates the middle piece (Fig. 20-B to I, inclusive). Their irregular distribution sometimes gives them the appearance of entwining the filament spirally (cf. Fig. 17), especially in the spermatozoa of the kite (*Milvus* sp.), the cuckoo (*Cuculus canorus*), the turkey (*Meleagris gallopavo*), the lesser black-backed gull (*Larus fuscus*), and the domestic pigeon, *Columba livia* (Retzius, 1909). The posterior limit of the middle piece is defined by the distal centriole, which migrates away from the proximal centriole (cf. Fig. 20-G, H, and I). In the chicken, the latter embeds itself in the nucleus, just inside the membrane, and divides into three granules (Retzius, 1909; Zlotnik, 1947). The greatly varying size of the middle piece enabled Retzius (1909) to list a few birds according to its increasing length, as follows: domestic mallard duck (*Anas platyrhynchos*), gull (*Larus fuscus*), lapwing (*Vanellus vanellus*), sandpipers (*Calidris alpina* and *Tringa ochropus*), woodcock (*Scolopax rusticola*), and domestic pigeon (*Columba livia*).

As to the cytoplasm, Scaccini (1942) noted that it gradually disappears during spermiogenesis in the guinea fowl (*Numida meleagris*), as if melting away. Guyer (1916) remarked that fragments of cytoplasm appear to be cut off from the transforming spermatid of the chicken. Retzius (1909) gave several illustrations in which a large mass of protoplasm is shown clinging to the head or to the middle piece of the immature spermatozoon of the mallard duck (*Anas platyrhynchos*) and the tufted duck (*Fuligula fuligula*), but he did not describe its ultimate disposition. Schöneberg (1913), who studied spermiogenesis in several species of ducks, merely stated that a similar vesicular mass of residual cytoplasm grows smaller and disappears.

For some time prior to their discharge from the testis, the spermatozoa are attached to a Sertoli cell, a nongerminal element that has migrated from the periphery of the seminiferous tubule. As soon as their heads have developed, a number of adjacent spermatozoa form a bundle and simul-



taneously bury themselves in the cytoplasm of a single Sertoli cell (Fig. 21). The spermatozoa of each bundle lie parallel, with their axial filaments directed toward the lumen of the tubule (cf. Fig. 16-B and C), although, at the start of their differentiation, their long axes lie in all directions. Loisel (1902) attributed the alignment of the spermatozoa to a chemical attraction exerted by a secretion of the Sertoli cell.

The spermatozoon is not entirely mature morphologically when it leaves the testis. In the sparrow (*Passer domesticus*), for example, the spiral filament of the tail cannot be distinguished until the spermatozoon is passing through the excurrent ducts (Loisel, 1902).

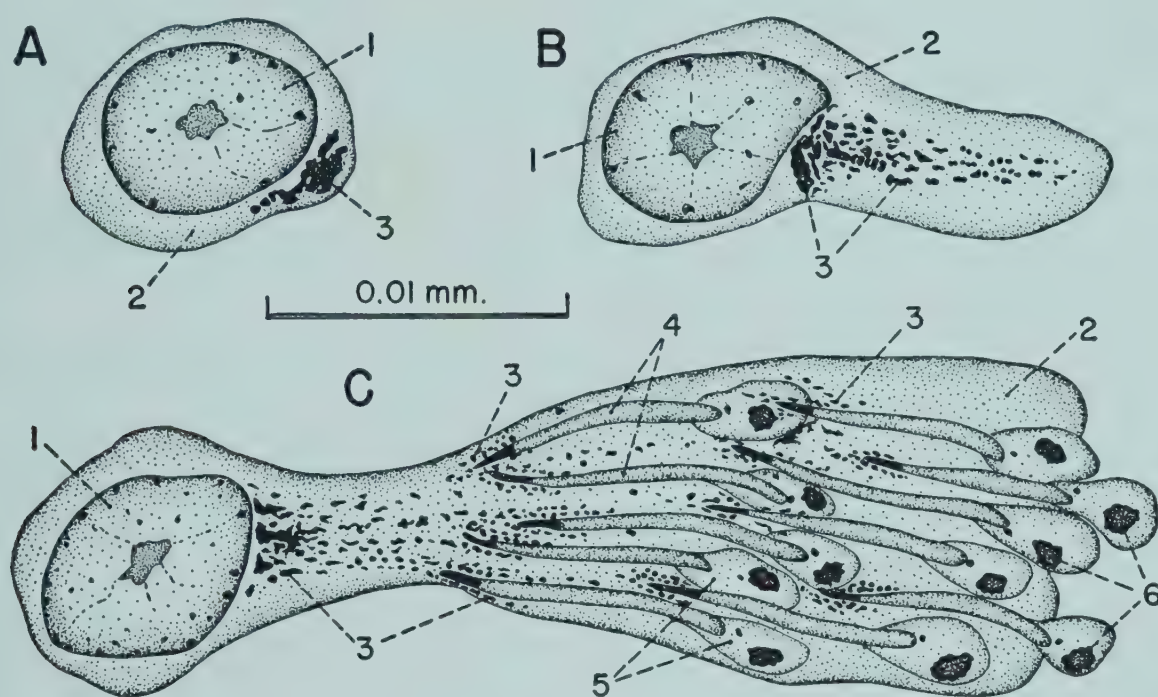


Fig. 21. Sertoli cells from the testis of the chicken (*Gallus gallus*), prepared to demonstrate the Golgi material. (Redrawn with modifications after Zlotnik, 1947.)

A, resting Sertoli cell; B, Sertoli cell at the stage when its cytoplasm begins to elongate; C, Sertoli cell containing a bundle of late spermatids. All in scale.

1, nucleus; 2, cytoplasm of Sertoli cell; 3, Golgi material of Sertoli cell; 4, heads of contained spermatids; 5, cytoplasm of contained spermatids; 6, Golgi material of contained spermatids.

Whenever a bundle of spermatozoa is detached from its Sertoli cell and passes into the lumen of the tubule, all the neighboring germinal elements are stimulated to kinetic activity that extends even to the spermatogonia. In this way, spermatogenesis proceeds discontinuously, as if by a series of relays.

### *The Motility of Spermatozoa*

Like the spermatozoa of other animals, avian sperm cells normally do not show motility within the testes. The capacity to move is gradually acquired during passage through the epididymides and vasa deferentia (Anderson, 1922; Munro, 1935, 1938c), a process which requires from 1 to 4 days in the chicken, depending upon mating activity (Munro, 1938c).

According to Ballowitz (1888), the spermatozoa of passerine birds are



propelled by a rapid oscillation of the tail, a motion which he likened to the vibration produced by plucking the string of a musical instrument. Simultaneously, the entire cell rotates. The rotation, Ballowitz believed, is an entirely passive movement caused by the corkscrew shape of the cell, especially its head.

The head of the chicken spermatozoon may be capable of independent movement to a certain extent (*Warren and Kilpatrick, 1929*). It is sufficiently curved to cause the spermatozoon to take a spiral course; the tail, however, appears to undulate rather than oscillate (*Ballowitz, 1888*).

The axial filament of the tail apparently supplies propulsive force through the contractile properties residing in the fibrils that compose it. The head acts as a stabilizing mechanism and gives direction to the movement of the cell. As Ballowitz (1888) observed, the tail moves in an aimless and spasmodic fashion when deprived of the head.

Makhovka (1939) found that chicken spermatozoa, like those of other vertebrates, proceed through a flowing liquid in a direction opposed to the current; that is, they exhibit rheotaxis.

If forced (by ligation of the ducts) to remain in the vasa deferentia, chicken spermatozoa may live and show motility for as long as 30 days. Since the cells remain viable in the ligated ducts equally as long if the testes are removed, the testis hormone apparently plays no part in promoting their survival (*Munro, 1938b*).

The motility of chicken spermatozoa may be affected by the bird's diet. According to Craft, McElroy, and Penquite (1926), cocks fed rations of poor quality produce a large percentage of immotile sperm cells.

There have been a number of studies of the factors influencing the degree and duration of motility of chicken spermatozoa *in vitro*. Temperature has been found to have a marked effect, whether the semen is diluted or undiluted. In diluted specimens, the motility of the cells depends not only upon temperature but also upon the composition, concentration, and pH of the diluting fluid and upon the extent of dilution.

In general, spermatozoa become gradually less active as the temperature is lowered. At about 1.7° C. (*Munro, 1938d*), spermatozoa in undiluted specimens are immobilized, but motility returns when the temperature rises again. The immobilization of sperm cells by low temperatures permits conservation of stored energy which is rapidly dissipated by active movement (*Munro, 1938d*). For this reason, the motility of spermatozoa stored *in vitro* persists for a period of time which is inversely proportional to the storage temperature, provided this is above 0° C. The data in the table (*Shaffner, Henderson, and Card, 1941*) indicate the relationship between storage temperature and the duration of sperm cell motility in undiluted cock (*Gallus gallus*) semen. It has also been stated that the optimum storage temperature is -2° C. (*Koch and Robillard, 1945*).



Storage Temperature (° C.)	Duration of Motility
37	4 hr.
28	28 hr.
6	7 days
0-1	14 days

Under special conditions, chicken spermatozoa may retain their motility after various periods of storage at temperatures below freezing. If semen is stored under a layer of mineral oil, cells may still be motile after 5 days at  $-2^{\circ}\text{C}$ . (*Koch and Robillard, 1945*). Immersion for 1 to 10 minutes in a freezing mixture at  $-15^{\circ}\text{C}$ . may fail to destroy the motility of all cells if the specimen is rewarmed in 30 minutes (*Winberg, 1941b*). Quick-freezing of semen (partially dehydrated by the addition of levulose) at  $-76^{\circ}\text{C}$ . has permitted spermatozoa to retain motility after 52 days of storage at the same temperature (*Shaffner, Henderson, and Card, 1941*). Spermatozoa have been found to retain complete motility for 10 weeks when diluted with Ringer's solution (containing 40 per cent glycerol) and quick-frozen at  $-79^{\circ}\text{C}$ . (*Polge, Smith, and Parkes, 1949*).

The ability of chicken sperm cells to retain their motility in undiluted semen stored at low temperatures appears to vary seasonally. At a storage temperature of  $2^{\circ}$  to  $3^{\circ}\text{C}$ ., motility persists for 7 to 8 days from November to April, inclusive, and for only 6 days during the remainder of the year (*Wheeler and Andrews, 1943*). Thyroid function possibly regulates the persistence and degree of motility, since either stimulation of the cock's (*Gallus gallus*) thyroid by the administration of thyroprotein (*Shaffner, 1948*) or its depression by treatment with thiouracil (*Shaffner and Andrews, 1948*) results in the production of sperm cells that are initially less motile than normal and that survive for only about 70 per cent of the usual time at  $4^{\circ}\text{C}$ .

When undiluted chicken semen is warmed from room temperature to  $41^{\circ}\text{C}$ ., the activity of spermatozoa continues or slightly increases. When diluted specimens are heated, the specific effect of certain diluents becomes apparent at  $35^{\circ}\text{C}$ . In normal saline, Ringer's solution, Baker's diluent, Milovanov's diluent, or fluid from either the infundibulum or the albumen-secreting region of the hen's oviduct, spermatozoa exhibit some loss of motility at  $35^{\circ}\text{C}$ . and are completely immobilized at  $41^{\circ}\text{C}$ ., although a subsequent drop in temperature restores normal activity. Motility is not decreased over the same temperature range when the suspending fluid is distilled water, chicken blood serum, sperm serum (the supernatant fluid from several pooled and centrifuged specimens of semen), or the fluid from the shell-secreting portion of the hen's oviduct (*Munro, 1938d*). In Ringer's solution, chicken sperm cells can withstand a temperature of



50° C. for 2 minutes before suffering an irreversible loss of motility (Winberg, 1941b).

The specific effect of different diluents is again apparent when spermatozoa are stored at any given temperature. The accompanying table gives the length of time chicken spermatozoa have been found to remain motile when semen is mixed with five volumes of various diluting fluids and held at 8° C. (Motohashi and Moritomo, 1927).

Diluent	Duration of Motility (hours)
Dextrose (6%)	9
Physiological saline (0.75%)	13
Whole egg albumen	15
Ringer's solution	27

Grodzinski and Marchlewski (1935) noted that the duration of motility of cock (*Gallus gallus*) spermatozoa is progressively shortened as the relative quantity of diluting fluid is increased. These authors observed that some motility persists in specimens diluted 1:10 with Tyrode's solution and stored 120 hours, but that it ceases within 46 to 72 hours in a 1:40 solution. Semen of the turkey (*Meleagris gallopavo*) can be diluted 1:400 with a balanced salt solution having a fructose concentration equal to that of normal semen (60 mg/%); spermatozoa retain motility up to 50 hours in this solution (Pace, Moravec, and Mussehl, 1952).

Ishikawa (1930) found that 0.9 per cent or 1.0 per cent physiological saline solution is the optimum concentration for making a 1:10 dilution of cock semen but considered egg albumen to be superior to this solution. It has been observed by Lorenz and Tyler (1951) that 0.033 M glycine in physiological saline as well as 0.25% solutions of various proteins will extend viability of cock spermatozoa in 1:20 dilution; part of the effect is evidently due to chelation of heavy metal ions in the diluent solution. According to Motohashi and Moritomo (1927), a storage temperature of 10° C. permits maximum duration of chicken sperm cell motility when whole egg albumen is used as a diluent. Duck (*Anas platyrhynchos*) spermatozoa suspended in the thin albumen of the hen's (*Gallus gallus*) egg are said to retain their motility longest at 16° C. (Grodzinski and Marchlewski, 1935).

The effect of the pH of the suspending medium on the duration of motility of chicken spermatozoa held at 2° C. is summarized in the table (Grodzinski and Marchlewski, 1935). Motility is apparently best preserved in a medium slightly more alkaline than semen, the average pH of which is from pH 7.2 (Zagami, 1937) to pH 7.5 (Parker, McKenzie, and Kempster, 1942b).



$pH$ of Suspending Medium	Duration of Motility (hours)
5.4	0.5
6.4	72.0
6.8	120.0
7.2	216.0
7.6–8.0	241.0

Grodzinski and Marchlewski (1938) investigated the merits of various serums as diluents of chicken semen. They found that motility persists almost as long in the serum of the pheasant (*Phasianus* sp.) or the guinea fowl (*Numida meleagris*) as in that of the hen, and that the serums of certain amphibians and fishes are better diluting fluids than those of mammals and reptiles. In pigeon (*Columba livia*) serum, motility ceases almost immediately. Cock serum causes spermatozoa to agglutinate, usually within 15 minutes, although Munro (1938*d*) apparently used it successfully. The serum of capons, on the other hand, has no agglutinating effect. Agglutination by cock serum may be prevented by heating either the serum or the seminal fluid, or by washing the spermatozoa, to which the agglutino-gen seems to adhere very weakly.

Johansson (1946), who exposed cock spermatozoa to X-rays (doses of 305 r to 24,400 r, with an intensity of 1,220 r per minute), observed that motility is unaffected until the dose exceeds 610 r. On the other hand, Kosin (1944*a*) found that spermatozoa are able to withstand treatment with 8,300 r of soft X-rays (delivered at the rate of 924 r per minute) without loss of motility, although their capacity for movement is distinctly reduced after the administration of higher doses (up to 36,900 r).

### *The Metabolism of Spermatozoa*

Certain aspects of the metabolism of cock spermatozoa *in vitro* have been investigated. Winberg (1939) found that the respiratory quotient of sperm cells is about 0.92. Dilution of the suspension, within certain limits, simultaneously increases the intensity of respiration and decreases longevity. Respiration and motility cease at the same time (Winberg, 1941*b*). This author also noted that spermatozoa have the ability to use certain sugars (glucose, fructose, mannose, and maltose), aerobically.

Kosin (1944*a*) observed that the rate of metabolic processes at 37.5° C. varies greatly in different specimens of semen. Oxygen consumption ranges from 1.68 to 5.03 cu. mm. of oxygen per hour per milligram (dry weight). After the addition of sodium succinate to the substrate, the range is from 2.68 to 6.42 cu. mm. per hour per milligram, the increase being due to the activity of succinoxidase in the spermatozoa. The amount of carbon dioxide evolved as the result of the anaerobic glycolytic function of spermatozoa may be from 8.16 to 22.32 cu. mm. per hour per milligram.



Winberg (1941a) estimated the cozymase content of a sperm cytolysate to be approximately 3 micrograms per-milligram of dry substance.

### Spermatogenesis in Hybrid Birds

Many observers are in agreement that spermatogenesis in hybrid birds may be fairly normal up to the first reduction division. At this point, it becomes aberrant to a varying extent.

Both Guyer (1900) and Smith (1912) noted that synapsis in the primary spermatocytes of hybrid pigeons (*Columba livia*) fails to lead to the formation of bivalent chromosomes, apparently because of incompatibility

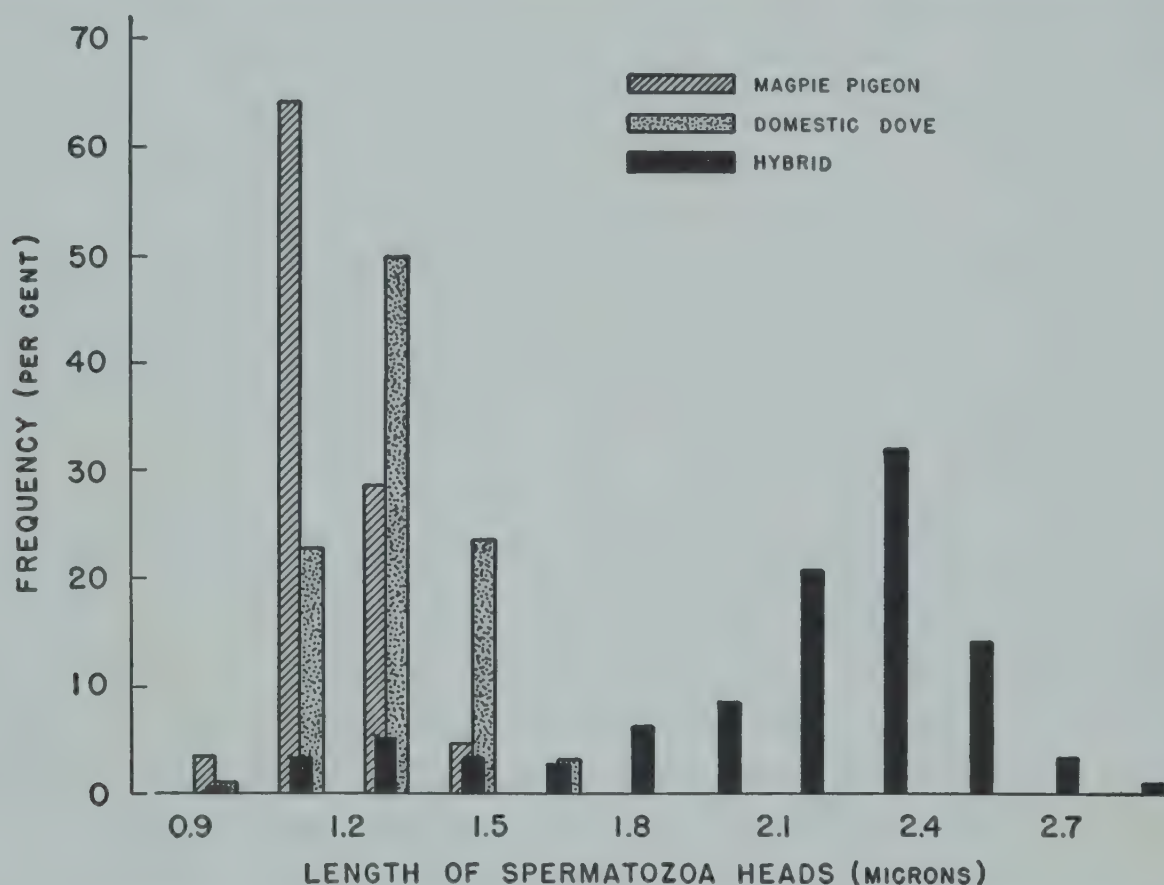


Fig. 22. Frequency distribution in the size of spermatozoa heads found in random samples of semen from magpie pigeons, domestic doves, and their hybrids, *Columba livia*. (Constructed from the data of Smith, 1912.)

between the maternal and paternal elements. The chromatin is often distributed at random and very unequally in the division of the primary spermatocytes, although sometimes (according to Guyer) two spindles are formed and the respective parental chromosomes are apparently thus segregated. Smith observed that the second reduction division frequently does not occur in hybrids of the domestic dove and the magpie pigeon (*Columba livia*); instead, the secondary spermatocytes become converted into giant spermatids which in turn are transformed into spermatozoa of approximately twice normal size (Fig. 22). Smith and Guyer both found that the heads of many spermatozoa are deformed by the presence of a beadlike varicosity resulting from the incomplete metamorphosis of the spermatid nucleus. A very high percentage of similarly deformed sperm cells was noted by Smith and Haig Thomas (1913) in a bird that was the offspring



of a female Reeves' pheasant (*Syrnaticus reevesii*) and a male Formosan pheasant (*Phasianus colchicus formosanus*). In hybrids of the ringdove (*Streptopelia decaocto*) and the pearlneck dove (*Streptopelia chinensis*), about 11 per cent of the spermatozoa may be of this type and 25 per cent of the type with elongated heads (Shrigley, 1940).

In wider crosses, such as that between the chicken and the guinea fowl, *Numida meleagris* (Guyer, 1912), or the pheasant, *Phasianus colchicus* (Cutler, 1918), spermatozoa are not produced. Guyer (1912), while studying spermatogenesis in offspring of a Black Langshan cock (*Gallus gallus*) and a guinea hen (*Numida meleagris*), observed a mass of primary spermatocytes in the contraction phase of synapsis and only a few cells in the spermatid stage. Cutler (1918) found that irregular clumps of chromatin, instead of bivalent chromosomes, are formed in the primary spermatocytes of hybrids of pheasant cocks (*Phasianus colchicus*) and Gold Campine hens (*Gallus gallus*), and that spermatogenesis proceeds no further.

Crew and Koller (1936) made a detailed study of spermatogenesis in the sterile hybrid of the Muscovy duck (*Cairina moschata*) and the mallard duck (*Anas platyrhynchos*). Although meiosis occasionally proceeds normally through the first maturation division, the resulting secondary spermatocytes degenerate. Frequently, the first reduction division produces binucleate secondary spermatocytes because of failure of the cytoplasm to divide; the nuclei in these cells undergo vacuolization and degeneration. Many spermatogonia with two nuclei are present. These cells eventually give rise to giant cells containing from two to eighteen nuclei and destined to dedifferentiate. Meiosis in all types of cells is characterized by many abnormal and irregular spindle formations, chromatid bridges, lagging bivalent chromosomes, and frequent loss of chromosomes into the cytoplasm. It was suggested that the cause of sterility is genotypic and that the chromosomes of the parental species may differ structurally although appearing to be similar. In the parents, genes responsible for normal gametogenesis are apparently complementary; in the hybrid, incompatibility leads to profound disturbances in cellular metabolism that affect the relation between karyokinesis and cytokinesis.

## FACTORS INFLUENCING GAMETOGENESIS IN BIRDS

The production of reproductive cells by both male and female birds is controlled by the endocrine system and primarily, it appears, by the pituitary gland. In the exercise of this control, there is a complex and not completely understood interaction between a number of different hormones.

In addition, certain extrinsic factors indirectly influence gametogenetic activity through their effect on the endocrine system. A few may have a direct effect on the gonads.



### The Age of the Bird

Most wild birds do not lay eggs or produce spermatozoa until they are approximately a year old. Domestic birds, especially chickens, reach sexual maturity when considerably younger, because they have been selected and bred for early reproductive activity. Pullets (*Gallus gallus*) begin to lay eggs at the age of 5 or 6 months, although the time of the year when they are hatched and various other factors may delay or accelerate the attainment of maturity (Romanoff and Romanoff, 1949, pp. 19–23). Observations on male Barred and White Plymouth Rocks, *Gallus gallus* (Hogue and Schnetzler, 1937; Kumaran and Turner, 1949a), Leghorns, and New Hampshires (Parker, McKenzie, and Kempster, 1942a) indicate that spermatogonial multiplication begins at the age of 4 to 6 weeks, and that the first spermatozoa form between the ages of 16 and 24 weeks. Occasionally, there is a precocious appearance of spermatozoa in the testes of cockerels 8 to 12 weeks old. The ring-necked pheasant (*Phasianus colchicus*), although a seasonal breeder, may also produce sperm cells at this age, in the autumn (Kirkpatrick and Andrews, 1944). Male turkeys (*Meleagris gallopavo*) usually reach maturity when about 7 months old (Parker, 1946b), but some individuals may mature at 4.5 months of age (Lorenz and Lerner, 1946).

### Season

In practically all wild birds, reproductive activity is a seasonal phenomenon, although its cyclical nature is more apparent and pronounced in some species than in others. Breeding occurs during the spring and early summer. The gonads then regress to a quiescent state and remain inactive until the approach of the breeding season of the following year. Domestic birds, of course, do not conform so obviously to the natural cyclical pattern of reproduction. Even in the rooster (*Gallus gallus*), however, there is a significant rise in spermatogenetic activity between December and April (Parker and McSpadden, 1943a; Wheeler and Andrews, 1943), and the spring increase in egg-laying by the hen (*Gallus gallus*) is well known (Romanoff and Romanoff, 1949, pp. 13–17).

The periodic activation of the gonads usually becomes apparent in wild birds during January, considerably in advance of the mating season (Fig. 23). The first indication of change is an increase in the size of the sex glands. Growth may proceed gradually at first and then become very rapid. In the starling (*Sturnus vulgaris*), for example, there is slow testicular and ovarian enlargement during February and March, followed by marked and sudden hypertrophy in April (Bissonnette and Zujko, 1936). In other species, such as the sparrow (*Passer domesticus*), the ovary does not become detectably larger until after the testes have begun to grow, and in some



females it may not increase greatly in size until the middle of May (Ringoen and Kirschbaum, 1939).

Ovarian enlargement is correlated with the growth of follicular ova, which however, do not increase in size at the same rate. Bissonnette and Zujko (1936) observed that the ovary of the starling (*Sturnus vulgaris*) contained three classes of follicles at the beginning of December: (1) those

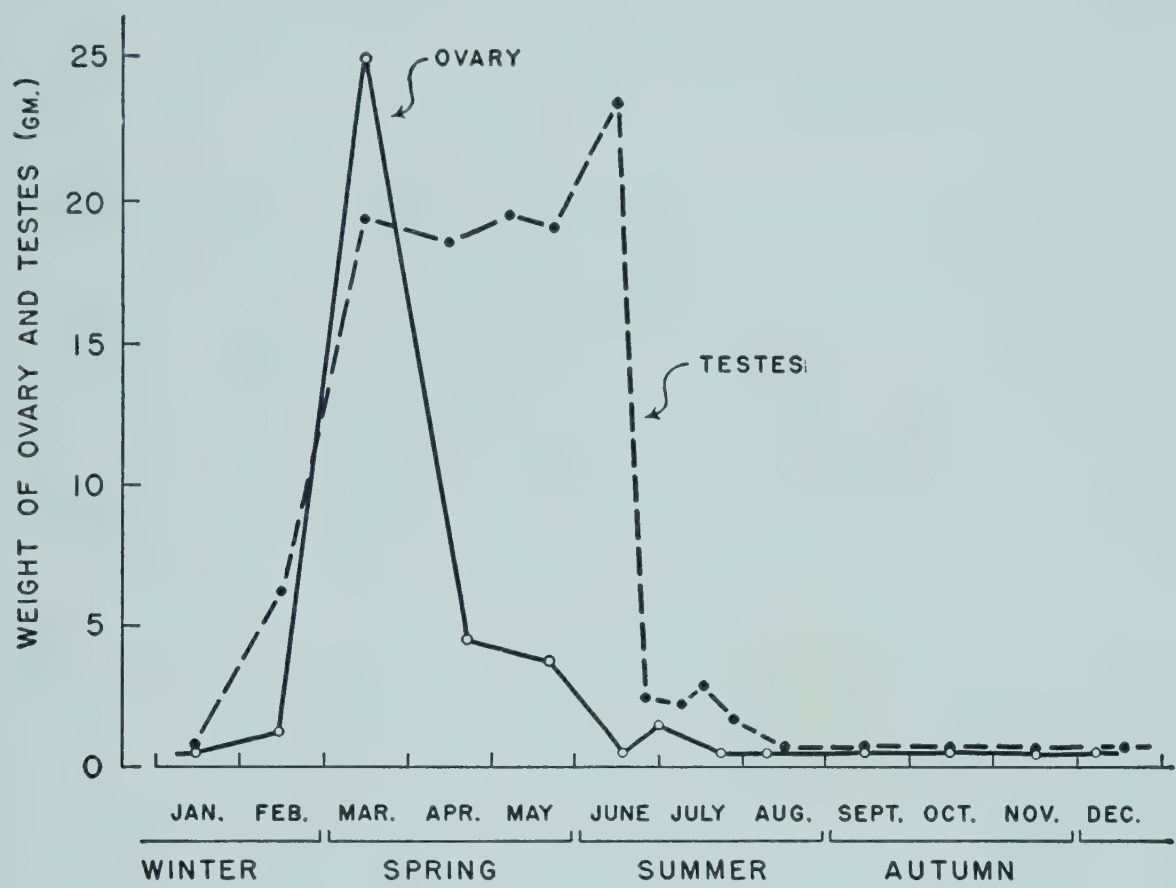
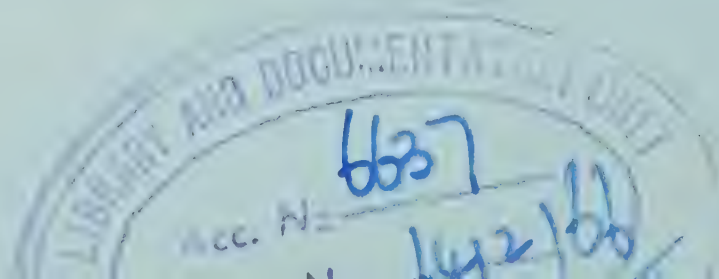


Fig. 23. Seasonal changes in the weight of the ovary and in the combined weight of the testes in adult mallard ducks, *Anas platyrhynchos*. (Reconstructed after Höhn, 1947.)

up to 0.04 mm. in diameter; (2) those from 0.04 to 0.4 mm. in diameter; (3) those from 0.4 to 0.54 mm. in diameter. From this time until April 20, the fourteen follicles of the third group grew at greatly different rates, so that their size varied over a continually wider range, as the table shows. The

Date	Range in Size (mm.)
Dec. 7	0.40-0.54
Feb. 11	0.62-0.99
Mar. 17	0.90-1.48
Apr. 3	0.99-1.83
Apr. 10	1.01-3.14
Apr. 20	0.94-9.08

three largest follicles considerably outstripped the others in size toward the end of the period of observation. About March 25, the largest follicle of all entered a phase of accelerated growth, about 26 days long, during which the average daily increment in diameter was nearly thirty-two times as large as





during the preceding 108 days. Shorter divisions of time, however, indicate that the growth rate of the largest follicle increased considerably on April 3 and again on April 10, as the accompanying data show. The deposition of

Period	Relative Growth Rate
Dec. 7-Feb. 11	1
Feb. 11-Mar. 17	2
Mar. 17-Apr. 3	3
Apr. 3-Apr. 10	27
Apr. 10-Apr. 20	85

globules or granules of “primordial yolk” (termed “white yolk” by these authors) apparently began on April 3, and growth during the last 10 days may be attributed to the accumulation of layered light and dark yellow yolk. In the pigeon (*Columba livia*), according to Riddle (1911), this last period begins only 5 to 8 days before ovulation, approximately at the same time as in the ring-necked pheasant (*Phasianus colchicus*), bobwhite quail, *Colinus virginianus* (Romanoff, 1943c), and chicken, *Gallus gallus* (Riddle, 1916; Romanoff, 1931).

The enlargement of the testes in the spring is phenomenal. During the nonbreeding season, these organs are minute and, in many species, weigh less than a milligram (Bergtold, 1926). The table gives the combined weights of the two testes of several American birds (found near Denver, Colorado) in both the resting and the active state (Bergtold, 1926). These data indicate that testicular increase may range from fifty-two times, in the

Species	Gonad Weight (gm.)	
	Resting	Active
Mourning dove ( <i>Zenaidura macroura</i> )	0.012	0.628
Long-crested jay ( <i>Cyanocitta stelleri</i> )	0.001	0.345
English sparrow ( <i>Passer d. domesticus</i> )	0.001	0.450
Virginia’s warbler ( <i>Vermivora virginiae</i> )	0.001	0.500
Robin ( <i>Turdus migratorius</i> )	0.011	1.140

mourning dove, to five hundred times, in the Virginia’s warbler. Bissonnette (1930a) noted that the size (volume) of the starling’s (*Sturnus vulgaris*) testes is fifteen hundred times as great in April as in November. Similar observations have been made regarding the white-crowned sparrow, *Zonotrichia leucophrys*, of the Pacific coast (Blanchard, 1941) and the Oregon junco, *Junco oreganus* (Wolfson, 1942).

Multiplication of the germinal elements in the testes is responsible for the greater part of this enlargement, for the interstitial cells between the seminiferous tubules are least numerous during the active period (Bissonnette, 1930a). The tubules increase in both diameter and length (Benoit and Ott, 1944). In the sparrow (*Passer domesticus*), the histological changes that produce testicular growth begin in January. At this time,



spermatogonia start to push into the matrix of the tubules (*Kirschbaum and Ringoen, 1936*). During February and the first half of March, the spermatogonia multiply and are transformed into spermatocytes (*Loisel, 1900; Foley, 1928*). Mature spermatozoa begin to form late in March (*Loisel, 1901; Foley, 1928; Kirschbaum and Ringoen, 1936; Riley, 1938*). The male starling (*Sturnus vulgaris*) apparently does not produce spermatozoa until April, in the vicinity of Hartford, Connecticut (*Bissonnette and Chapnick, 1930*). In the mallard drake (*Anas platyrhynchos*), free spermatozoa may be present in the testes as early as February 1, near London, England (*Höhn, 1947*).

*Bissonnette (1930b)*, who studied the regressive changes in the starling's (*Sturnus vulgaris*) testes, noted that involution begins as early as June. It proceeds at a rate that varies with the individual bird. The germ cells disappear from the walls of the seminiferous tubules in the reverse order of their appearance, since the most mature cells degenerate first and no new ones are formed to replace them. The testes reach their minimal size in November and December in this species (*Bissonnette, 1930a*), and probably in most others.

Regressive changes in the ovary can be observed in those domestic fowl which still undergo prolonged pauses in laying. As a laying period comes to an end, the growth of the larger oöcytes is retarded. Degeneration of most follicles exceeding 2 mm. in diameter occurs after laying has ceased. In most modern breeds of chicken (*Gallus gallus*), however, new follicles begin to develop before the old ones have completely degenerated (*Ritter, 1940*).

### Light

The seasonal nature of reproductive activity in birds is due to the fact that the principal extrinsic factor affecting the gonads is light, acting through the mediation of the anterior pituitary gland, or hypophysis (*Benoit, 1935c*). Activation of the ovary and testes, occurring soon after the winter solstice, not only coincides with but is dependent upon the lengthening of the days. Even in domestic hens (*Gallus gallus*), there is a clear parallel between egg production and the amount of diurnal light throughout the year (*Whetham, 1933*).

When the influence of light was first pointed out by *Rowan (1925)*, who worked with the junco (*Junco hyemalis*) it was suggested that longer daylight periods operated by increasing physical activity and general metabolism and thus indirectly affected the gonads (*Rowan, 1928*). The importance of light *per se* has subsequently been almost conclusively established through experiments on birds of both sexes and many species, including the starling, *Sturnus vulgaris* (*Bissonnette, 1930a, 1931a*), the sparrow, *Passer domesticus* (*Kirschbaum, 1933; Ivanova, 1935; Kendeigh, 1941*), the mourning dove, *Zenaidura macroura* (*Cole, 1933*), the white-eye, *Zosterops*



*palpebrosa japonica* (Miyazaki, 1934), the duck, *Anas platyrhynchos* (Benoit, 1934, 1935a, 1935b), the blue jay, *Cyanocitta cristata* (Bissonnette, 1939), ring-necked pheasants (*Phasianus colchicus*), the bobwhite quail (*Colinus virginianus*), and the ruffed grouse, *Bonasa umbellus* (Bissonnette and Csech, 1936; Clark, Leonard, and Bump, 1936, 1937). Since 1925, when it was discovered that all-night lights increased the egg production of hens, *Gallus gallus* (Kennard and Chamberlin, 1931), the use of artificial light to lengthen the day has become a widespread method of stimulating egg-laying.

It is interesting to note that the female is less responsive to the stimulus of light than the male, at least among wild birds. Additional illumination has been found to hasten sexual maturity in female domesticated and semidomesticated birds, such as the ring-necked dove, *Streptopelia decaocto* (Cole, 1933), the turkey, *Meleagris gallopavo* (Albright and Thompson, 1933; Scott and Payne, 1937; Charles, Wilcox, Flagg, and Tepper, 1938; Wilcke, 1939), and the chicken, *Gallus gallus* (Svetosaroff and Streigh, 1940; Callenbach, Nicholas, and Murphy, 1944). By contrast, lengthened days produce a limited response in female starlings, *Sturnus vulgaris* (Burger, 1942), and sparrows, *Passer domesticus* (Riley and Witschi, 1938a; Ringoen and Kirschbaum, 1939; Riley, 1940). Although some follicles may grow and the oviduct may enlarge, ovulation does not occur. It is suggested that, in these birds, some psychological stimulus, such as pairing and nest-building, is necessary to augment the effect of light, which apparently can initiate oöcytic development but cannot sustain it (Burger, 1942).

The testes of wild birds, however, are extremely sensitive to variations in the diurnal light ration. This must exceed a certain minimum level to promote full spermatogenesis (Burger, Bissonnette, and Doolittle, 1942); in the starling (*Sturnus vulgaris*), this minimum is about 12.5 hours (Burger, 1940). It is possible to manipulate the condition of the starling's testes at will by varying the total daily amount of light received by the bird (Bissonnette, 1931a; Burger, 1939).

The gonadal response to light varies also according to the intensity of illumination. In the starling (*Sturnus vulgaris*), for example, light-stimulated spermatogenetic activity is directly proportional to light brightness, provided this is suboptimal (Bissonnette, 1931b). Bronze turkey (*Meleagris gallopavo*) hens produce their maximum number of eggs when light with an intensity of 2 foot-candles is used to extend the day, and remain unaffected when a light weaker than 0.1 foot-candle is employed (Asmundson, Lorenz, and Moses, 1946).

The gonads are sensitive to qualitative as well as quantitative differences in light stimuli. Burger (1943) observed that light must be of wave lengths between 0.58 micron and 0.68 micron to stimulate the starling (*Sturnus vulgaris*) to produce spermatozoa. Benoit and Ott (1944) subjected Pekin



drakes (*Anas platyrhynchos*) to illumination of seven different wave lengths between 400 and 700 millimicrons and found that growth of the testes is greatest under red and orange-red light (wave length from 600 to 700 millimicrons), as shown in Fig. 24. The effect of yellow light is marked and that of green light is fairly strong. Extreme red produces little growth and violet-blue even less; infrared rays elicit no response at all. These findings are in general agreement with the results of experiments on starlings, *Sturnus vulgaris* (Bissonnette and Wadlund, 1931), male and female sparrows, *Passer domesticus* (Bissonnette, 1932; Ringoen, 1942), and Narra-

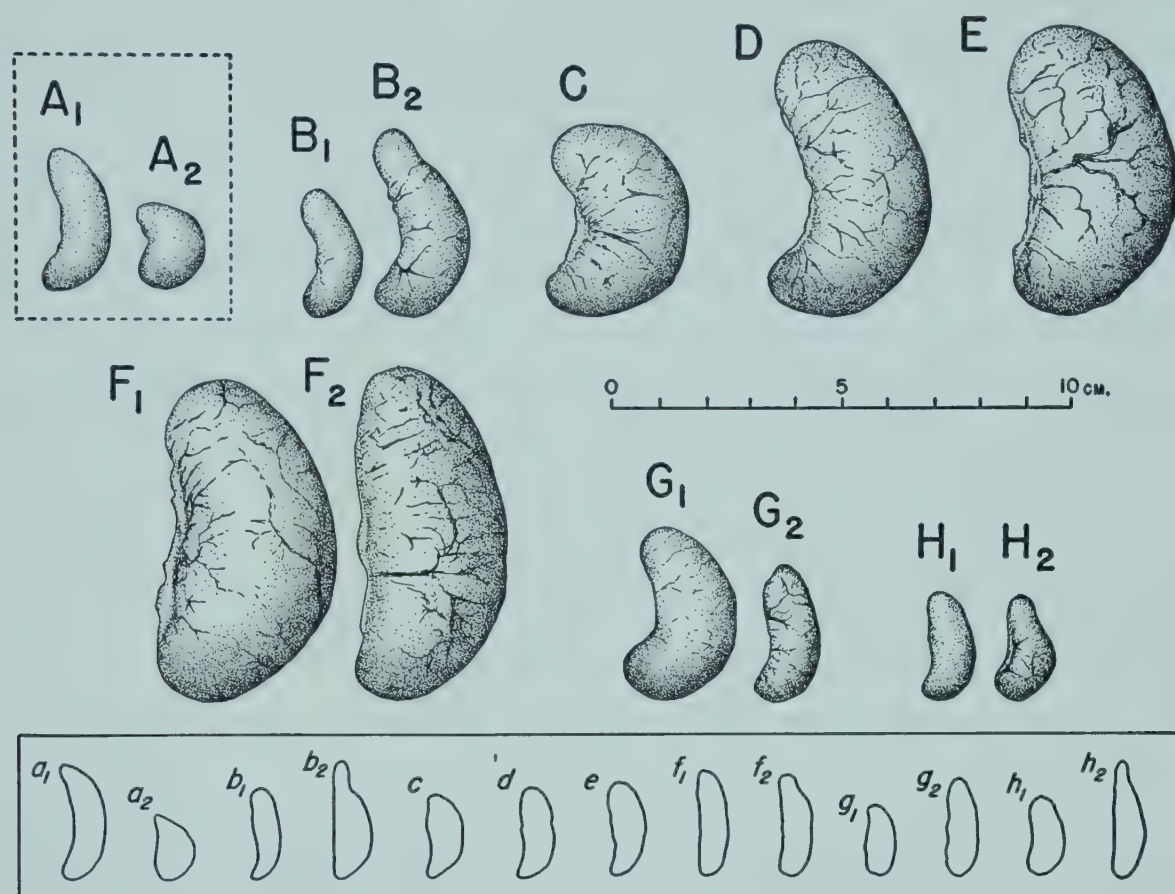


Fig. 24. The size of the left testis of the Pekin duck (*Anas platyrhynchos*) when the bird is irradiated with light of different wave lengths. (Redrawn with modifications after Benoit and Ott, 1944.)

Insert: A<sub>1</sub>, A<sub>2</sub>, the testes of birds receiving no irradiation.

B<sub>1</sub>, B<sub>2</sub>, testes from birds irradiated with blue light; C, with green light; D, with yellow light; E, with orange-red light; F<sub>1</sub>, F<sub>2</sub>, with red light; G<sub>1</sub>, G<sub>2</sub>, with extreme red light; H<sub>1</sub>, H<sub>2</sub>, with infrared light. All in scale.

Insert: a<sub>1</sub> to h<sub>2</sub>, show the original sizes of the irradiated organs.

gansett turkey hens, *Meleagris gallopavo* (Scott and Payne, 1937). In drakes (*Anas platyrhynchos*), however, violet-blue radiations are as effective as red when conducted directly to the hypophysis by a quartz rod. This finding suggests that there are encephalic light receptors which are sensitive to violet-blue but which normally do not receive it because of its absorption by the intermediate living tissues (Benoit and Ott, 1944).

Normally, gonadal response to light probably depends on the reception of stimuli by the eye (Benoit, 1935b, 1938; Ringoen and Kirschbaum, 1937, 1939). It appears, however, that neither the retina nor the optic nerve is an



essential pathway for the transmission of stimuli to the hypophysis, for the effect of light on the gonads is still observed after the removal of the eyeball (Benoit, 1935e) and severance of the optic nerve (Benoit, 1935d). As Benoit and Ott (1944) showed, the tissues separating the hypophysis from the orbit are sufficiently thin to permit the passage of light of certain wave lengths. These authors therefore suggested two mechanisms which light may stimulate: (1) an oculohypophyseal photostimulation mechanism, and (2) an encephalohypophyseal photostimulation mechanism, more deeply seated. It may be added that experiments on pigeons (*Columba livia*) have failed to demonstrate myelinated nerve fibers connecting the optic tract and the hypothalamico-hypophyseal fasciculus, but the possibility of a nonmyelinated pathway is not ruled out (Drager and Baker, 1944).

In spite of the influence of light, regression of the testes of wild birds often sets in before the natural day has reached its maximum length (Bissonnette, 1930a); and the peak of egg production by domestic hens (*Gallus gallus*) also occurs before this time. Furthermore, migratory birds of the northern hemisphere winter in the southern hemisphere, where they are exposed to long days, without a recrudescence of sexual activity (Kirschbaum and Ringoen, 1936; Rowan, 1938). Additional illumination in September has failed to induce testicular enlargement in adult male sparrows (*Passer domesticus*), although it proved effective on juvenile birds (Riley, 1936b, 1937). Oögenesis and spermatogenesis may even be initiated during the 8-hour days of January in wild birds such as the robin, *Erithacus rubecula* (Marshall, 1952). These facts perhaps indicate the existence of an inherent sexual rhythm that has become synchronized with the seasons (Riley, 1936b, 1937). Wolfson (1954) finds that long days promote sexual activity in the junco (*Junco hyemalis*) and white-crowned sparrow (*Zonotrichia leucophrys*); regression of gonads occurs regardless of length of day employed in the experiment, although a period of short days reconditions the testes, permitting repetition of the cycle as many as five times in one year. Experiments have shown also that spontaneous involution of the starling's (*Sturnus vulgaris*) testis occurs if artificially lengthened days are continued for a sufficiently long period of time. It has therefore been suggested that refractoriness to light stimulation is eventually and normally established (Burger, 1947). Possibly the gonad-stimulating mechanism becomes exhausted by the continuous action of light, and the ability to respond is not restored until the natural day is too short to provide an effective stimulus. Evidence that it is the pituitary and not the gonad that becomes refractory has been presented by Miller (1948, 1949), who worked with the golden-crowned sparrow (*Zonotrichia atricapillus*).

Experiments on photoperiodism in birds do not in general permit a distinction between the effect of a long light period and that of a short dark period. Kirkpatrick and Leopold (1952) have demonstrated that the



bobwhite quail (*Colinus virginianus*) shows no reproductive activity when kept on a 10-hour day for 37 days beginning in January, but will reach full sexual activity in the same time with a total of 10 hours of light provided that the dark period be interrupted for 0.5 to 1 hour; the birds respond to 12.5 hours, but not to 14 hours of continuous darkness. There is an inverse relation between the weight of the gonads and length of the maximum dark period.

Night interruption will also induce sexual maturation in juncos (*Junco hyemalis*) and white-throated sparrows, *Zonotrichia albicollis* (Jenner and Engels, 1952), and Farner, Mewaldt, and Irving (1953) have demonstrated that more frequent interruption of the dark period increases the response of the white-crowned sparrow (*Zonotrichia leucophrys*) with respect to gonad weight and stage of spermatogenesis. The data of the later investigators (Farner, Mewaldt, and Irving, 1953) are given in the accompanying table.

Daily Exposure to Light	Weight of Testes (milligrams)	Spermatogenesis
Continuous 10 hours	1.2	None
“ 12 “	3.9	Beginning
“ 18 “	190.0	Complete
Interrupted for 1.5 hours in each 10 hours	8.2	None
“ “ 1 minute in each 0.5 hour	87.0	Partial
“ “ 1 hour in each 2 hours	170.0	Complete

The parakeet, *Melopsittacus undulatus*, may be an exception to the generalization that light stimulates sexual activity in birds; fledgling males reach maturity when isolated in complete darkness, showing a greater increase in weight of gonads than birds kept under natural lighting conditions. Constant illumination shortens the period of sexual activity (Vaugien, 1953).

Hormones of the Hypophysis and Other Glands

Intrinsically, the activity of the bird’s gonads are controlled by the secretions of the hypophysis, or pituitary gland. This gland, stimulated by light, elaborates its hormones, which in turn act upon the ovary or testes.

The importance of the hypophysis is indicated by the observation that its removal has resulted in testicular atrophy in many birds, including chickens (*Gallus gallus*), turkeys (*Meleagris gallopavo*), pigeons, *Columba livia* (Hill and Parks, 1934), and Rouen drakes, *Anas platyrhynchos* (Benoit, 1935f). Furthermore, the administration of either whole pituitary extracts or of purified “follicle-stimulating” hormone of the hypophysis causes follicular growth in the ovary and enlargement of the testes. The ovarian response has been seen in ring-necked doves (*Streptopelia decaocto*) and pigeons (Riddle and Flemion, 1928; Riddle and Polhemus,



1931; Riddle and Bates, 1933), hens (Clark, L. N., 1915; Gutowska, 1931; Bates, Lahr, and Riddle, 1935) and sparrows, *Passer domesticus* (Riley and Witschi, 1938a, 1938b); the testicular reaction has been observed in immature drakes, *Anas platyrhynchos* (Schockaert, 1931), Brown Leghorn (*Gallus gallus*) cockerels (Domm and Van Dyke, 1932a; Schockaert, 1933), Silver King squabs (Evans and Simpson, 1934), pigeons (*Columba livia*), and ring-necked doves, *Streptopelia decaocto* (Lahr, Riddle, and Bates, 1936). The serum of pregnant mares contains a pituitary-like gonadotropin and has a similar effect on hens (Fraps and Riley, 1942), male sparrows (Riley, 1937; Pfeiffer, 1947), female sparrows (Riley and Witschi, 1938b; Pfeiffer and Kirschbaum, 1941), immature male chickens (Asmundson and Wolfe, 1935; Breneman, 1936; Hamburger, 1936), immature male turkeys (*Meleagris gallopavo*), and female chickens (Asmundson and Wolfe, 1935). Negative results have been obtained by Sluiter and Oordt (1947) in treating cockerels with the mare serum preparation and the same authors (1949) note that enlargement of the testis of the chaffinch (*Fringilla coelebs*) in response to the drug is not accompanied by spermatogenesis.

The hypophyseal hormone which affects the gonads contains two fractions, the "follicle-stimulating" or gonadotropic fraction and the so-called luteinizing fraction. In birds, there is possibly a third fraction whose function is allied to that of the luteinizing fraction (Nalbandov, Meyer, and McShan, 1951). As shown in experiments with immature male chickens, the gonadotropic fraction causes enlargement of the tubules, and the luteinizing fraction produces hypertrophy of the interstitial tissue (Breneman, 1936; Nalbandov, Meyer, and McShan, 1946), which secretes the male sex hormone (Kumaran and Turner, 1949c). In chickens of both sexes, the germinal and nongerminal elements of the gonad are affected by pregnant-mare serum (Asmundson and Wolfe, 1935; Breneman, 1936; Asmundson, Gunn, and Klose, 1937).

The follicle-stimulating hormone, as its name implies, induces growth of follicular ova in female birds (Bates, Lahr, and Riddle, 1935). Also, like pregnant-mare serum, it indirectly leads to enlargement of the oviduct (Riddle and Flemion, 1928; Asmundson and Wolfe, 1935; Bates, Lahr, and Riddle, 1935) because the stimulated ovary secretes the female sex hormone (Allen, Whitsett, Hardy, and Kreibert, 1924), which in turn brings the oviduct into reproductive condition (Riddle and Tange, 1926; Keck, 1934; Asmundson, Gunn, and Klose, 1937; Ringoen and Kirschbaum, 1939; Ringoen, 1940; Pfeiffer and Kirschbaum, 1941).

The function of the luteinizing hormone in the female is apparently the control of ovulation. The injection of the luteinizing fraction into laying hens may cause ovulation several hours prematurely (Fraps, Olsen, and Neher, 1942; Fraps, Riley, and Olsen, 1942). Its effect on the first follicle



of the hen's laying cycle is much more pronounced than on any of the subsequent follicles. Possibly the time of ovulating and of laying the ova from the latter follicles is affected by some principle of the ruptured but still unresorbed follicles (*Rothchild and Fraps, 1944; Fraps, 1946*), although resorption is very rapid, especially within the first few days after ovulation (*Davis, 1942; Romanoff, 1943c*).

In the female chicken, the effect of gonadotropic hormones varies according to the age and the laying condition of the bird. Such hormones induce ovarian growth in the immature chick (*Domm and Van Dyke, 1932b*), but they cannot cause ova to enter the period of rapid yolk formation until the bird is close to maturity (*Asmundson and Wolfe, 1935; Asmundson, Gunn, and Klose, 1937*). Apparently there are metabolic hormones, or other principles, that are important in yolk deposition (*Nalbandov and Card, 1946*). In nonlaying hens and high-producing hens alike, the follicle-stimulating fraction causes the simultaneous growth of many ova, the ovulation of which is prevented unless the luteinizing hormone is given. Probably a hormonal imbalance is created and the production of the bird's own luteinizing principle is thus inhibited. In low-producing hens, however, the follicle-stimulating fraction produces the normal series of ova of graded sizes, the largest of which ovulates without the artificial intervention of the luteinizing fraction (*Nalbandov and Card, 1946*).

Female ring-necked doves (*Streptopelia decaocto*), pigeons, *Columba livia* (*Riddle and Flemion, 1928; Riddle and Polhemus, 1931*), and sparrows, *Passer domesticus* (*Pfeiffer and Kirschbaum, 1941*), when immature, are also partially or entirely unresponsive to gonadotropic hormones. As the breeding season approaches, the sparrow becomes less refractory. Ovulation and normal egg-laying may then be induced (*Riley and Witschi, 1938b*); and, in fact, the inactive ovary can be so overstimulated that many follicles grow simultaneously and are ovulated (*Pfeiffer and Kirschbaum, 1941*). In the adult ring-necked dove's ovary about twenty-six ova enlarge instead of the usual two or three (*Riddle and Bates, 1933*).

The effect of injected gonadotropic preparations, like that of light, is considerably less marked in the female than in the male, at least in certain species (*Riddle and Flemion, 1928; Riddle and Polhemus, 1931; Asmundson and Wolfe, 1935; Riley and Witschi, 1938a*). Apparently the gonad-hypophysis mechanism is much more difficult to activate in the female.

Certain hormones not of pituitary origin appear to have a limited or indirect influence on the activity of the reproductive cells. One of these hormones is androgen, secreted by the interstitial cells of the testis. When injected into the male, androgen stimulates spermatogenesis if the testis is already beginning to elaborate this hormone spontaneously (*Pfeiffer, 1947*) and if secondary spermatocytes have developed (*Kumaran and Turner, 1949e*). Otherwise, androgen depresses the hypophysis (*Burger, 1944*), and



the resulting lack of the gonadotropic hormone causes the involution of spermatocytes, but not of spermatogonia (Kumaran and Turner, 1949e). It is suggested that androgen supports the maturation of the sex cells (Burger, 1945), starting with the transformation of secondary spermatocytes into spermatids (Kumaran and Turner, 1949e).

Injection of estrogen into the male inhibits production of spermatocytes, presumably by depressing secretion of pituitary gonadotropin; spermatogenesis can be restored by thyroprotein or sulfamethazine, but not by testosterone (Kumaran and Turner, 1949b). Administration of progesterone to cocks (*Gallus gallus*) prevents the appearance of mature spermatozoa in the testis (Baldissera, 1954). The growth of spermatogonia appears to be autonomous and independent of the pituitary gonadotropic hormone, which probably controls the growth and division of the primary spermatocytes alone (Kumaran and Turner, 1949e).

The thyroid hormone may also play a role. Experiments on chickens (Crew, 1925; Wheeler and Hoffmann, 1948c; Kumaran and Turner, 1949d) and mallard drakes, *Anas platyrhynchos* (Jaap, 1933), indicate that thyroid feeding increases testis weight and promotes spermatogenesis. The thyroid hormone is thought (Kumaran and Turner, 1949d) to affect the male gonad through its influence on the secretion of the luteinizing fraction of the pituitary hormone, which stimulates the production of androgen. However, both Shaffner (1948) and Huston and Wheeler (1949) failed to influence spermatogenesis in chickens by the administration of thyroprotein.

Thyroid preparations augment egg production in the hen (Turner, Irwin, and Reineke, 1945), especially during summer (Turner, Kempster, Hall, and Reineke, 1945). However, they reduce the size of the mature ovum (Asmundson, 1931; Asmundson and Pinsky, 1935). Probably because they increase the metabolic rate (Riddle, Hollander, McDonald, Lahr, and Smith, 1945), the level of serum vitellin, which limits deposition of yolk in the final growth phase, is sharply decreased by thyroxin treatment (Clavert, 1952). Blivaiss and Domm (1942a, 1942b) showed that thyroidectomy delayed sexual maturity in Brown Leghorn chickens (*Gallus gallus*) of both sexes, although Benoit (1937) observed that the effect of thyroid removal was gradually overcome, with time, in Pekin drakes (*Anas platyrhynchos*).

Adrenaline appears to have an unfavorable influence on the gonads. Its injection has been found to affect the production of spermatozoa very adversely in the domestic cock, *Gallus gallus* (Wheeler, Searcy, and Andrews, 1942), and the sparrow, *Passer domesticus* (Perry, 1941).

Cortisone induces precocious spermatogenesis in cockerels (*Gallus gallus*) and Pekin ducks, *Anas platyrhynchos* (Leroy, 1952).

The pituitary gland itself secretes another hormone, prolactin, which



is antagonistic to gametogenesis. Prolactin inhibits the secretion of the gonadotropic hormone in both sexes (*Bates, Riddle, and Lahr, 1937*). In the female pigeon (*Columba livia*) prolactin prevents yellow yolk formation (*Bates, Riddle, and Lahr, 1937*), causes ovarian regression (*Bates, Lahr, and Riddle, 1935*), and induces broodiness (*Riddle, 1938*). When administered to the male, it brings about testicular atrophy (*Lahr, Riddle, and Bates, 1935; Nalbandov, Hochhauser, and Dugas, 1945*).

The gonadotropic function of the hypophysis can also be inhibited by exposure of the bird's head to X-rays. Such treatment reduces the egg production of ring-necked pheasants, *Phasianus colchicus* (*Clark and Bump, 1944*).

### Time of Day

The cellular divisions of spermatogenesis apparently do not take place at a uniform rate throughout the day. Riley (*1936a, 1937*) found that they normally occur in the sparrow (*Passer domesticus*) between 2:00 A.M. and 4:00 A.M. They may be induced during the day, however, by inversion of the light period or by reducing the bird's body temperature. Correspondingly, spermatogenesis may be partially suppressed at night if the bird is forced to exercise. Physical inactivity (*Foley, 1928*) and low body temperature (*Riley, 1936a, 1937*) therefore seem to be necessary for the actual cellular changes that lead to the production of spermatozoa. External temperature, however, is not a factor in spermatogenesis (*Benoit, 1935a*), although the effectiveness of artificially lengthened days in promoting testicular activity appears to be greater at a temperature close to that of the bird's body than at lower temperatures (*Burger, 1948*).

In male domestic chickens (White Leghorns, *Gallus gallus*), the difference between the cellular activity of day and of night appears to be less than in the sparrow (*Passer domesticus*). Riley (*1940*) was unable to find complete absence of cell divisions at any hour, but he noted that the level of spermatogenesis is higher at night than during the day, reaching a maximum at 3:00 A.M. Macartney (*1942*), on the other hand, counted a significantly larger number of dividing cells between 1:30 P.M. and 1:30 A.M. than during the morning hours, and suggested that the time of feeding is one of the factors controlling diurnal spermatogenesis in the chicken.

The feeding time appears to exert considerable influence on the hour of ovulation in the hen. Fowl exposed to a 24-hour day may adhere to a normal laying (and therefore ovulating) schedule until they are fed at night (*McNally, 1940; Fraps, Neher, and Rothchild, 1947*); they then lay their eggs at night instead of during the day. The same factors that regulate the hours of activity and body temperature appear to determine also the hours during which the ovum ripens and is released.



### Nutrition

The influence of diet on egg-laying by the domestic hen has received considerable attention. It is well known that egg production (and therefore oögenesis and yolk deposition) is dependent to a very great extent upon the quality and quantity of food consumed by the laying bird. Proteins, minerals, and vitamins are especially important (*Romanoff and Romanoff, 1949, pp. 38-47*).

Experiments indicate that diet also affects spermatogenesis. Reduced food consumption curtails the production of spermatozoa by Rhode Island Reds, *Gallus gallus* (*Parker and McSpadden, 1943a, 1943b*). In the pigeon (*Columba livia*), starvation causes the germinal elements to disappear from the testis, the amount of degeneration being proportional to the degree of inanition (*Marrian and Parkes, 1928*).

Deficiency of the B-complex vitamins leads to testicular degeneration in the pigeon (*Columba livia*). Thiamine appears to be more important in testis nutrition than riboflavin (*Marrian and Parkes, 1928*).

In the chicken, prolonged deficiency of vitamin E (the so-called reproduction vitamin) may cause involutionary changes in the testes, with progressive degeneration of the cellular elements in reverse order to that of their formation. In comparison with other animals, however, the male chicken appears to be extremely resistant to the lack of this vitamin (*Adamstone and Card, 1934b*), and can also continue to produce spermatozoa for 6 or 7 months on a diet inadequate in vitamin A (*Parker and McSpadden, 1941*). Minerals, such as magnesium, iron, copper, manganese, iodine, and bromine, appear to be more effective than vitamins in restoring normal spermatogenesis in cocks (*Gallus gallus*) fed an inadequate diet (*Alibrandi, 1940*).

Perry (1938) found that a positive gonadal response is elicited in sparrows (*Passer domesticus*) when they are fed irradiated wheat. This response becomes pronounced as soon as the preliminary exposure of the wheat to ultraviolet light exceeds 300 hours.

### Heredity

It has been shown beyond dispute that the number of eggs produced by domestic chickens is under hereditary control to a great extent. The high egg-laying records of modern flocks have been attained largely through selective breeding. It is equally possible to develop family lines characterized by very low egg production. The egg-laying capacity of a hen appears to be correlated with that of both her dam and her sire's dam (*Hall, 1935*).

Comparisons of spermatogenetic activity in males from strains of chickens of high and low fecundity, respectively, indicate that the rate of sperm cell



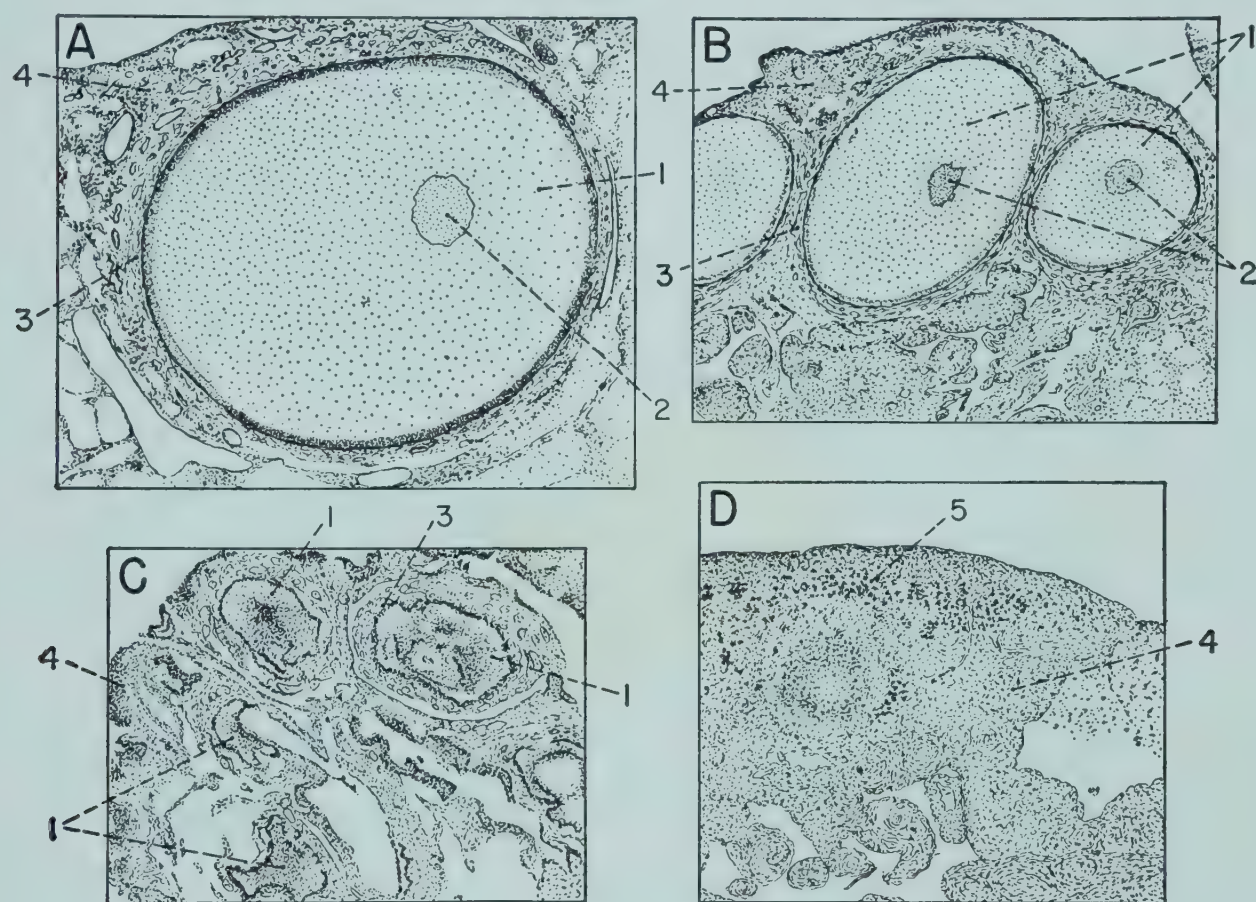
formation is under the influence of heredity equally as much as is the rate of egg formation (*Jones and Lamoreux, 1942*).

### Experimental Procedures

Experiments have shown that gametogenesis can be profoundly affected by certain factors that do not normally come into play. Among these are X-rays and various drugs.

#### *Effect of X-rays*

A number of investigators have studied the effect of X-rays on gametogenesis by irradiating chick embryos and the gonadal region of young chicks and of adult birds of both sexes.



**Fig. 25.** Effect of X-rays on the ovary of the chicken, *Gallus gallus*. (A, redrawn with modifications from Nalbandov and James, 1949; B, C, and D, from Essenberg and Karrasch, 1940.)

A, normal appearance of oöcyte about 0.6 to 0.7 mm. in diameter, and of follicle surrounding it ( $\times 50$ ); B, oöcytes with shrunken nuclei from a bird treated with 400 r of X-rays ( $\times 50$ ); C, effect of 2176 r ( $\times 50$ ); D, effect of three doses totaling 4200 r ( $\times 100$ ).

1, oöcyte; 2, nucleus of oöcyte; 3, follicle; 4, ovarian tissue; 5, pigment.

Female germ cells exhibit greater sensitivity to X-rays than male, regardless of the dosage or the period in the life cycle when treatment is administered. Essenberg and Zikmund (1938) found that ovaries of newly hatched chicks contain a reduced number of germ cells when as small a dose as 80 r has been given previous to the start of incubation. Many areas in the ovary are devoid of oöcytes when a dose of 200 r to 400 r is administered on



the fifth to seventh day of incubation. The latter range of dosages, when given to hatched chicks 7 to 21 days old, produces extensive atrophy and degeneration of ova. Essenberg and Karrasch (1940), who irradiated mature hens, noted that a dose of 400 r is sufficient to cause the nuclei of many ova to shrink (Fig. 25-B) and that doses ranging from 800 r to 1200 r produce marked changes, with atrophy of cytoplasm and nuclear contents. Higher doses, up to 2176 r, result in extreme atrophy of the ovular contents and collapse of many entire follicles (Fig. 25-C). These follicles assume the form of a crescent and their contents gradually disappear. After cumulative doses amounting to 4200 r, nuclei are found in the last stages of degeneration and the ovarian tissue is largely replaced by connective tissue (Fig. 25-D).

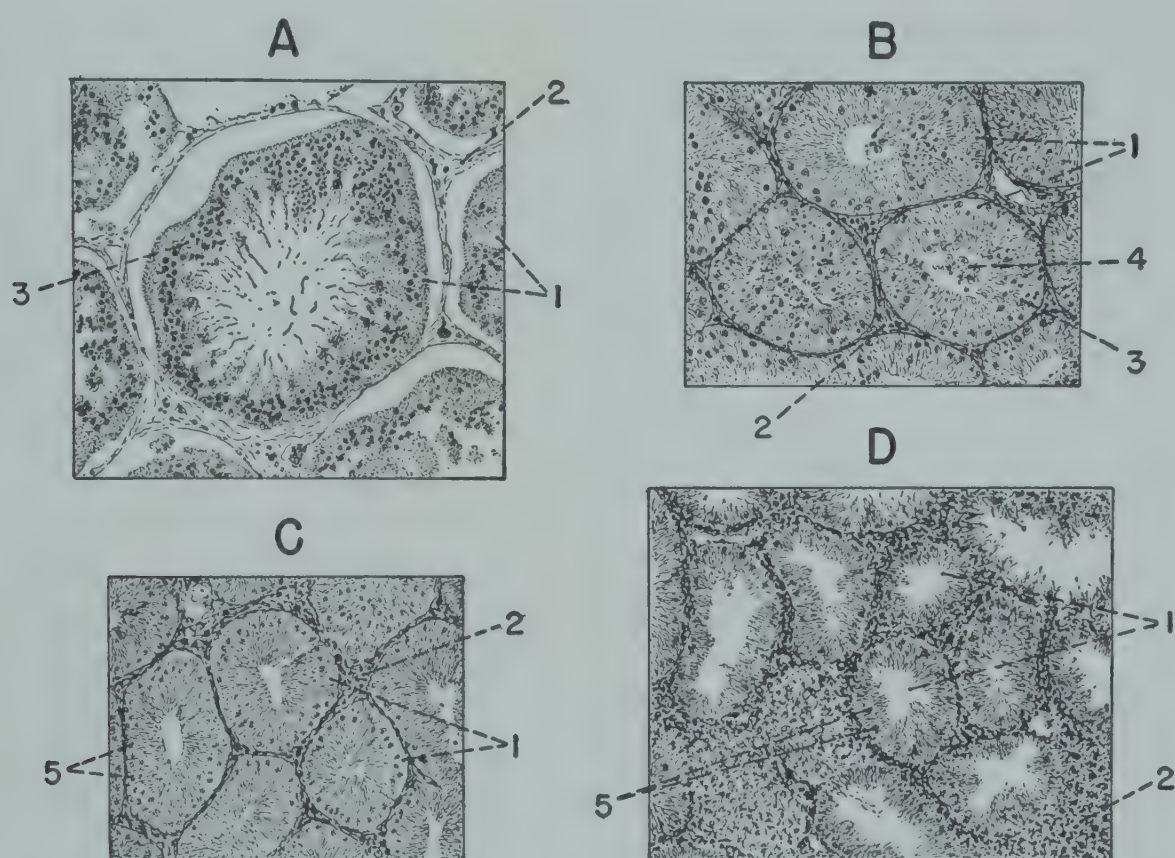


Fig. 26. The effect of X-rays (about 3600 r) on spermatogenesis in the male chicken, *Gallus gallus*. (Redrawn with modifications from Mirskaia and Crew, 1931.)

Sections of testes show: A, normal spermatogenesis in an untreated bird; B, diminished spermatogenesis 14 days after treatment; C, increased interstitial tissue and absence of all but lining cells in the tubules 25 days after treatment; D, lack of regeneration of spermatogenic tissue 4 months after treatment. All  $\times 80$ .

1, seminiferous tubule; 2, interstitial tissue; 3, zone of spermatogenesis; 4, degenerate spermatozoa; 5, lining cells.

By contrast, male germ cells appear to be affected little or not at all when the embryo is subjected, before incubation, to X-ray doses ranging from 40 r to 480 r, or when doses of 100 r to 400 r are given during incubation. However, if hatched male chicks less than 3 weeks old are irradiated with dosages in excess of 200 r, the division of spermatogonia is arrested, the nuclei of these cells become pycnotic, and the cytoplasm is pale (Essenberg and Zikmund, 1938). In adult cocks (*Gallus gallus*), a dose of



1276 r is required for initial injury, which is revealed by pycnosis of the nuclei in primary and secondary spermatocytes and by a decrease in the number of spermatids. After doses of 2176 r to 2816 r, pycnotic nuclei are also found in spermatids and in some spermatogonia, there is fragmentation of the cytoplasm, and spermatozoa are not present (*Essenberg and Karrasch, 1940*). When X-rays are administered before puberty, a dose of 2100 r is the minimum that prevents subsequent spermatogenesis in most individuals (*Sturkie, Pino, Weatherwax, Donnelly, and Dorrance, 1949*). Within 14 days after administration of a sterilizing dose, degenerate spermatozoa may be seen in the lumina of many tubules (Fig. 26-B). After 25 days the amount of interstitial tissue has increased (Fig. 26-C), and only one layer of cells remains lining the tubules (*Benoit, 1924; Mirskaia and Crew, 1931; Essenberg and Karrasch, 1940; Sturkie, Pino, Weatherwax, Donnelly, and Dorrance, 1949*). No regeneration of spermatogenic tissue occurs (Fig. 26-D).

### *Effect of Drugs*

Belenjkii (1940) found absence of spermatogenesis and increased amounts of interstitial tissue in the testes of cockerels (*Gallus gallus*) that had received injections of antitestis rabbit serum for 30 days, beginning at the age of 50 days.

Subcutaneous injections of sodium bromide given to chickens every day for 8 months (starting on the ninth day after hatching) appear to have an adverse effect on the testes, which become atrophic and infantile. The ovary seems to be affected little, if at all (*Borgatti, 1947*).

Injection of 24 micrograms of colchicine directly into the testis of the mature chicken causes severe degeneration of the seminiferous tissue within a week. Regeneration follows within 21 days after treatment (*Jenkins and Bohren, 1949*).

When sulfamethazine is given to week-old male chicks and administration continued for 4 weeks enlargement of the seminiferous tubules results and many spermatocytes may be seen dividing (*Asplin and Boyland, 1947*).

## THE CHROMOSOMES OF AVIAN REPRODUCTIVE CELLS

As already remarked, the mature reproductive cells of birds, like those of other animals, contain only half the number of chromosomes found in the immature sex cells and in all other cells of the embryo and the adult. When the nuclei of the ovum and the spermatozoon unite, the full complement of chromosomes (the diploid number) is restored.

In every species, the diploid number of chromosomes presumably is characteristic and constant. Unfortunately, the technical difficulties of counting chromosomes are so great that virtually all estimates are subject



TABLE 1  
The Diploid Number of Chromosomes  
in Some Representative Birds of Several Families

Species	Number of Chromosomes	Investigator
(Rheidae)		
<i>Rhea americana</i>	42-68	Shiwago and Peschkowskaja (1936)
(Casuariidae)		
<i>Dromaeus novae-hollandiae</i>	40-76	Shiwago and Peschkowskaja (1936)
(Alcidae)		
<i>Brachyramphus marmoratum</i>	50	Oguma (1937)
<i>Lunda cirrhata</i>	50	Oguma (1937)
(Hydrobatidae)		
<i>Oceanodroma leucorhoa</i>	74	Oguma (1937)
(Phalacrocoracidae)		
<i>Phalacrocorax carbo</i>	70	Oguma (1937)
(Anatidae)		
<i>Aix sponsa</i>	16	Schöneberg (1913)
<i>Cairina moschata</i>	16	Schöneberg (1913)
" "	34-62	Sokolovskaia (1935b)
" "	72	Crew and Koller (1936)
" "	80	Yamashina (1942)
<i>Anas platyrhynchos</i>	16	Schöneberg (1913)
" "	43-49	Alikhanian (1936)
" "	34-62	Sokolovskaia (1935b)
" "	48-69	Crew and Koller (1936)
" "	76-77	Werner (1927)
" "	79-80	Oguma (1938)
" "	80	Yamashina (1942)
<i>Aythya ferina</i>	16	Schöneberg (1913)
<i>Anas penelope</i>	16	Schöneberg (1913)
(Laridae)		
<i>Sterna albifrons</i>	66	Oguma (1937)
<i>Larus argentatus</i>	66	Oguma (1937)
(Phasianidae)		
<i>Coturnix coturnix japonica</i>	44	Kobayashi (1937)
" " "	78	Oguma (1938)
<i>Phasianus colchicus</i>	52-61	Unger (1936b)
" "	41-62	Trofimoff and Tiniakoff (1933)
<i>Chrysolophus amherstiae</i>	66-76	Scaccini (1939a)
<i>Chrysolophus pictus</i>	66-76	Scaccini (1939a)
<i>Gennaeus nycthemerus</i>	66-76	Scaccini (1939a)
<i>Gennaeus nycthemera beli</i>	66-76	Scaccini (1939a)
<i>Syrnaticus reevesii</i>	66-76	Scaccini (1939a)
<i>Gennaeus swinhoei</i>	66-76	Scaccini (1939a)
<i>Gallus gallus</i>	12	Loyez (1906); Sonnenbrodt (1908); Lécaillon (1910e)
" "	18	Guyer (1916)
" "	15-19	Guyer (1909b)
" "	18-20	Cutler (1918)
" "	30-32	Akkeringa (1927)
" "	32	Shiwago (1924)
" "	30-34	Hance (1923)



TABLE 1 (*Continued*)  
The Diploid Number of Chromosomes  
in Some Representative Birds of Several Families

Species	Number of Chromosomes	Investigator
<i>Gallus gallus</i>	35-36	Hance (1926 <i>b</i> )
“ “	36	Goldsmith (1928)
“ “	36-38	Akkeringa (1927)
“ “	29-39	Saguchi (1930)
“ “	35-40	Hance (1924)
“ “	36-40	Boring (1923)
“ “	33-44	Homedes i Ranquini and Martin de Frutos (1937)
“ “	42-44	Akkeringa (1927)
“ “	44-61	Unger (1936 <i>b</i> )
“ “	65-66	White (1932)
“ “	50-70	Scaccini (1937)
“ “	60-70	Hance (1926 <i>a</i> ); Popoff (1933)
“ “	32-71	Sokoloff and Trofimoff (1932, 1933)
“ “	73-74	Suzuki (1930)
“ “	77-78	Oguma (1938); Yamashina (1944)
“ “	76-80	Miller (1938)
<i>Pavo cristatus</i>	36-58	Tiniakoff (1934)
<i>Numida meleagris</i>	17	Guyer (1909 <i>a</i> )
“ “	66	Scaccini (1939 <i>a</i> )
<i>Meleagris gallopavo</i>	46	Shiwago (1929)
“ “	66-71	Scaccini (1939 <i>a</i> )
“ “	76-77	Werner (1931)
<i>Tetrastes bonasia</i>	86	Yamashina (1952)
(Columbidae)		
<i>Columba livia</i>	16	Guyer (1900, 1903); Harper (1904); Smith (1912)
“ “	32-42	Homedes i Ranquini and Martin de Frutos (1937)
“ “	50	Hance (1932)
“ “	61-62	Oguma (1927)
“ “	62-74	Painter and Cole (1943)
<i>Streptopelia decaocto</i>	16	Guyer (1900, 1903)
“ “	61-66	Painter and Cole (1943)
“ “	66	Tange and Nakahara (1939)
(Psittacidae)		
<i>Melopsittacus undulatus</i>	42	Jentsch (1935)
“ “	50-60	Crew and Lamy (1935)
(Turdidae)		
<i>Turdus pilaris</i>	74-81	Pogossianz (1937)
<i>Turdus merula</i>	60-85	Unger (1936 <i>b</i> )
(Fringillidae)		
<i>Acanthis cannabina</i>	64-85	Unger (1936 <i>b</i> )
(Ploceidae)		
<i>Passer domesticus</i>	22	Harper (1912)
“ “	40-48	Pogossianz (1937)
(Corvidae)		
<i>Corvus monedula</i>	56-67	Pogossianz (1937)



to revision (*Painter and Cole, 1943*). Many avian chromosomes are extremely small and easily escape detection even in sections showing the equatorial plate of the metaphase, where they are seen most clearly. Furthermore, these small chromosomes tend to clump together, unless the tissue is fixed less than one minute after the bird is killed (*Matthey, 1934*). Table 1 gives chromosome counts for a number of birds as determined from observations of both somatic and reproductive cells. It shows that

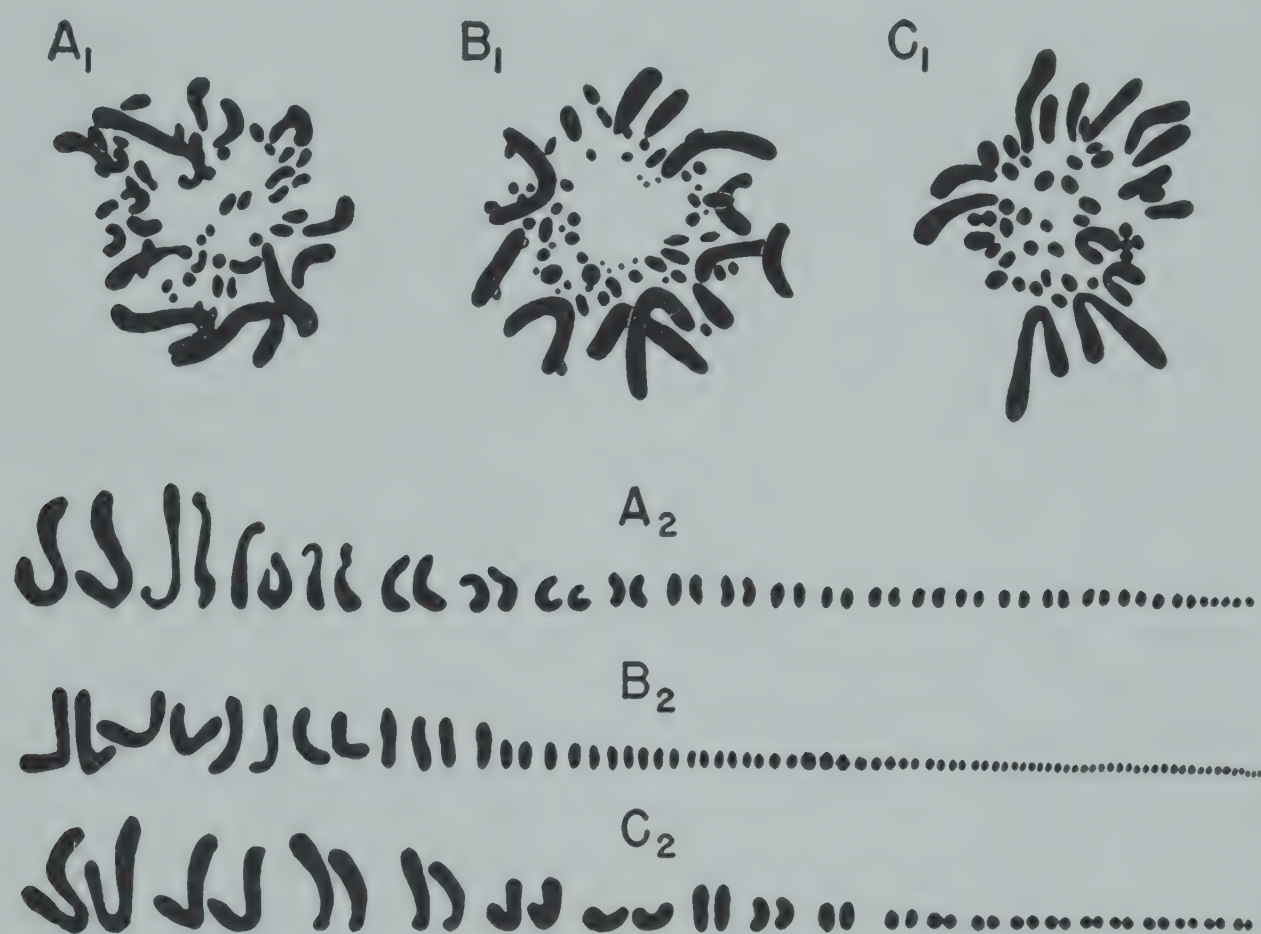


Fig. 27. The diploid chromosome complex in three (male) birds, as seen in equatorial plates and after being arranged according to size. (Redrawn with modifications A, after Shiwago, 1929; B, after Werner, 1927; C, after Tiniakoff, 1934.)

A<sub>1</sub>, A<sub>2</sub>, 23 pairs of chromosomes from the turkey (*Meleagris gallopavo*); B<sub>1</sub>, B<sub>2</sub>, 38 pairs of chromosomes from the Indian Runner duck (*Anas platyrhynchos*); C<sub>1</sub>, C<sub>2</sub>, 20 pairs of chromosomes from the peacock (*Pavo cristatus*). All  $\times 1500$ .

there is considerable disagreement between the findings of different investigators and also indicates that many workers have obtained such wide variation in their own results that they have reported only minimum and maximum figures. The data on the chicken, the species most frequently studied, show that improved techniques made it possible to count over six times as many chromosomes in 1938 as in 1906.

Avian chromosomes are characterized by an unusually great range in size (*White, 1932*). This fact may be seen in Fig. 27, which pictures the chromosomes of three species. In most birds, it is possible to distinguish three chromosome groups, as follows: (1) a rather small number of large bodies in the form of J's, V's, and rods; (2) a few medium-sized and short rods of gradually diminishing size; (3) many very small spherical elements



(Werner, 1927, 1931; Alikhanian, 1936; Crew and Koller, 1936; Oguma, 1937, 1938; Painter and Cole, 1943). The largest chromosomes are only a few microns in length; for example, about 3 microns in the chicken (Akerling, 1927); 5 microns in the pheasant, *Phasianus colchicus* (Scaccini, 1939a, 1939b). In equatorial plates of the metaphase, the largest chromosomes are arranged peripherally and the smallest occupy the central portion of the figure (Fig. 28).

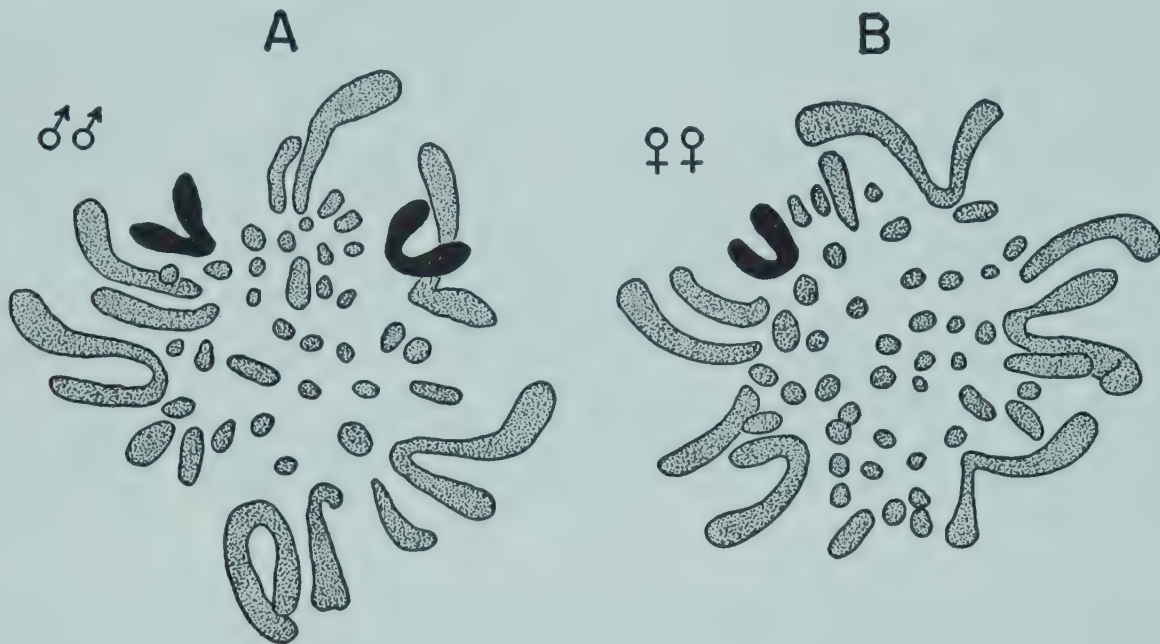


Fig. 28. The general complement of chromosomes in the chicken (*Gallus gallus*) as they appear in equatorial plate. (Redrawn with modifications after Sokoloff, Tiniakoff, and Trofimoff, 1936.)

A, the diploid set of chromosomes found in the cells of male birds; B, the diploid set of chromosomes found in cells of female birds (note that the V-shaped fifth largest element, shown in black, is in the haploid state).

It appears that some, at least, of the large chromosomes (macrochromosomes) are common to many species. A set of five chromosomes, for example, is found in each of seven gallinaceous birds; these are the elements designated as A, C, F, K, and N in Fig. 29 (Sokoloff, Tiniakoff, and Trofimoff, 1936). Of the fifteen largest forms identified in these birds, the remaining ten are distributed in various combinations, some of the ten being absent in each species. Again, it has been shown that three passerine birds—the sparrow (*Passer domesticus*), the jackdaw (*Corvus monedula*), and the thrush (*Turdus philomelos*)—also possess several chromosomes corresponding to certain elements in this series of fifteen (Pogossianz, 1937). In general, the largest body (a V-shaped form with arms of unequal length) is most consistently present. However, Oguma (1937) noted that all the elements are rod-shaped in Leach's petrel (*Oceanodroma leucorhoa*). Oguma also observed that, in several species of sea birds, the total number of chromosomes seems to vary inversely with the number of V-shaped elements.

Newcomer and Brant (1954) have pointed out that the microchromo-



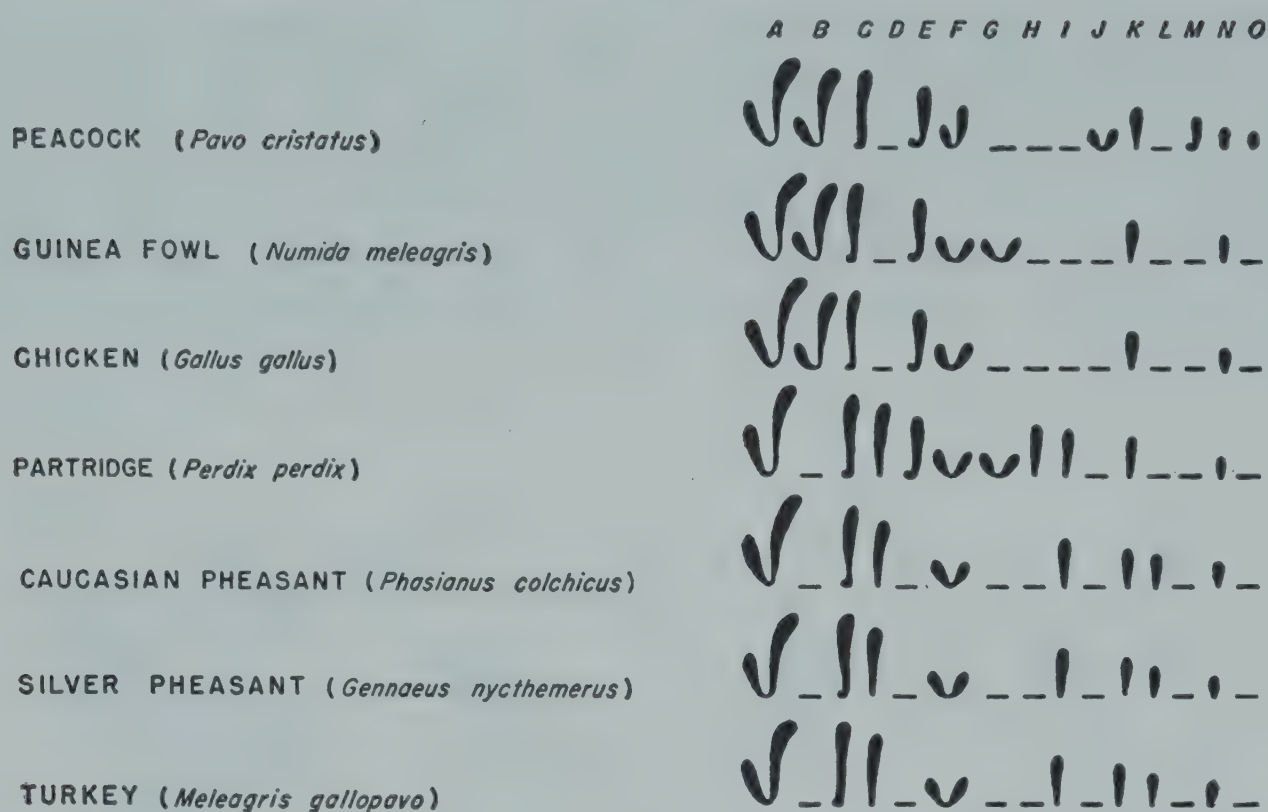


Fig. 29. Fifteen large chromosomes found in gallinaceous birds, and their distribution in seven species. (Redrawn with modifications after Sokoloff, Tiniakoff, and Trofimoff, 1936.)

somes resemble supernumerary chromosomes found in other organisms; they are variable in number, tend to fragment or fuse, and often divide precociously in both mitosis and meiosis. In view of this anomalous behavior and considering also the genetic evidence that there are only six linkage groups in the chicken, the authors suggest that the microchromosomes may have little importance in hereditary structure of *Aves*.

### The Sex Chromosome

Many breeding experiments performed early in the twentieth century indicated that, in *Aves*, the female and not the male is heterogametic for the sex-determining chromosome (*Boring and Pearl, 1914*). Later, cytological studies substantiated the genetical evidence. It is now fairly well established that all avian spermatozoa contain the so-called sex chromosome, whereas this element is absent from half the eggs of every female. The sex-determining mechanism thus falls into the simple XX-XO type (*Hance, 1924, 1932; Oguma, 1927; White, 1932; Popoff, 1933; Scaccini, 1937*). Eggs containing the X chromosome, when fertilized, give rise to male embryos with the XX chromosome complex; eggs lacking the X element produce female embryos, in whose cells the single X chromosome is of paternal origin (Fig. 30).

Although the preponderance of evidence is in favor of the XX-XO formula, it has been suggested that the sex-determining mechanism in the chicken, *Gallus gallus* (*Shiwago, 1924; Akkeringa, 1927*), the zebra parakeet, *Melopsittacus undulatus* (*Jentsch, 1935*), and the turkey, *Meleagris*



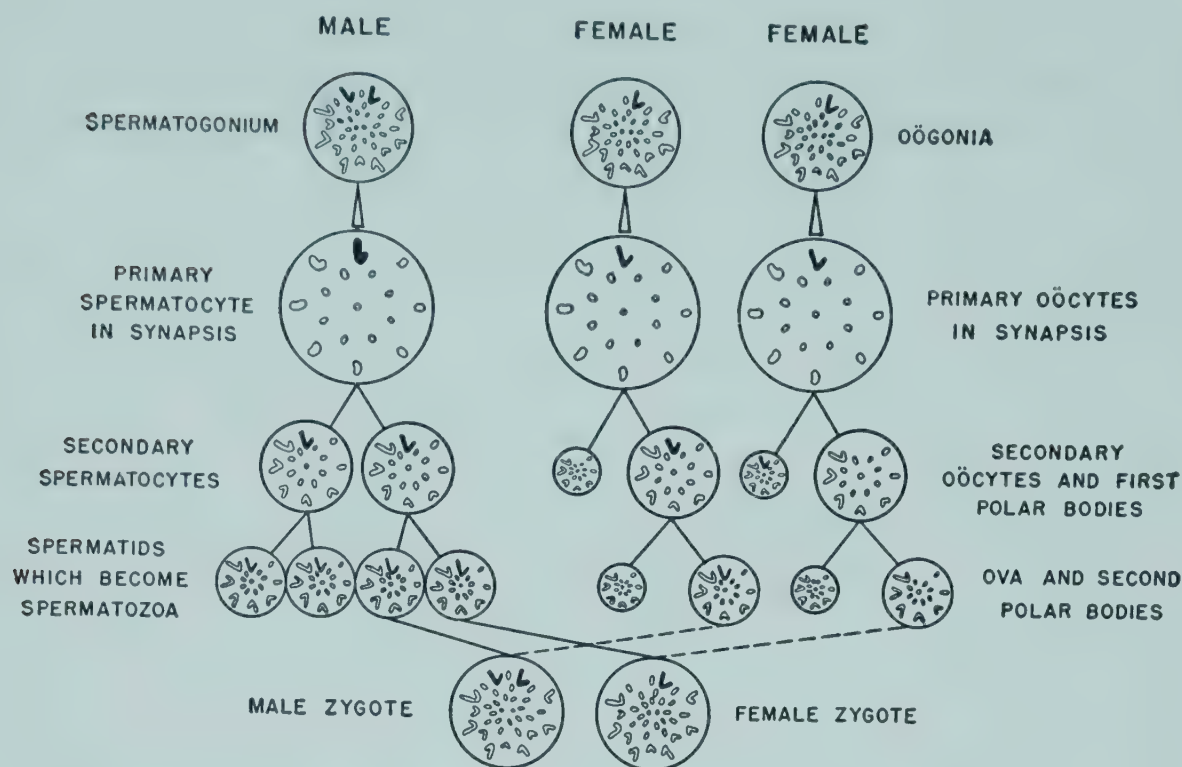


Fig. 30. Diagram of the behavior of avian chromosomes during the maturation of the germ cells and the fertilization of the ovum. The sex chromosome is indicated in solid black.

*gallopavo* (Shiwago, 1929), is of the XX-XY type; that is, that the X chromosome in the female is paired with a small Y chromosome, so that half the mature ova contain X and half Y. Werner (1927, 1931) proposed a more complex formula, ZZ-ZWs (or XX-XXy), for the Indian runner duck (*Anas platyrhynchos*) and the turkey (*Meleagris gallopavo*). Such a mech-

Species	Size Rank of Sex Chromosome	Investigator
Zebra parakeet ( <i>Melopsittacus undulatus</i> )	Second	Jentsch (1935)
Jackdaw ( <i>Corvus monedula</i> )	Second or third	Pogossianz (1937)
Turkey ( <i>Meleagris gallopavo</i> )	Fourth	Sokoloff, Tiniakoff, and Trofimoff (1936)
Caucasian pheasant ( <i>Phasianus colchicus</i> )	Fourth	Sokoloff, Tiniakoff, and Trofimoff (1936)
Silver pheasant ( <i>Gennaues nycthemerus</i> )	Fourth	Sokoloff, Tiniakoff, and Trofimoff (1936)
Ring-necked pheasant ( <i>Phasianus colchicus</i> )	Fourth	Unger (1936b)
Ring-necked dove ( <i>Streptopelia decaocto</i> )	Fourth	Painter and Cole (1943)
Blackbird ( <i>Turdus merula</i> )	Fourth	Unger (1936b)
Thrush ( <i>Turdus philomelos</i> )	Fourth or fifth	Pogossianz (1937)
Linnet ( <i>Acanthis cannabina</i> )	Fifth	Unger (1936b)
Peacock ( <i>Pavo cristatus</i> )	Fifth	Sokoloff, Tiniakoff, and Trofimoff (1936)
Partridge ( <i>Perdix perdix</i> )	Fifth	Sokoloff, Tiniakoff, and Trofimoff (1936)
Guinea fowl ( <i>Numida meleagris</i> )	Sixth	Sokoloff, Tiniakoff, and Trofimoff (1936)



anism, postulating two supplementary sex chromosomes in the ovum, met the requirements of this author's observation (apparently erroneous) that the cells of the female contained one more chromosome than those of the male.

The identity of the sex chromosome has been the subject of considerable investigation. In the chicken (cf. Fig. 28), it is apparently a medium-sized, V-shaped element (Suzuki, 1930), the fifth largest in size (Sokoloff, Tinia-koff, and Trofimoff, 1936; Unger, 1936b; Miller, 1938), rather than the V-shaped chromosome of largest size singled out by a number of workers (Hance, 1923, 1924, 1926b; Akkeringa, 1927; White, 1932; Scaccini, 1937). In most other species studied, the sex chromosome appears to correspond in appearance to the fifth chromosome of the chicken, but may not hold the same rank in size. The table gives the findings for various birds. There is little agreement regarding the sex chromosome of ducks (*Anas platyrhynchos*). Crew and Koller (1936) concluded that it is the V-shaped largest chromosome; Sokolovskaia (1935b) identified it as the rod-shaped third largest, and Oguma (1938) as the fifth, which is also rod-shaped. In the pigeon (*Columba livia*), the sex chromosome is possibly the largest element (Oguma, 1927; Hance, 1932), but it is also thought to be the fourth largest (Painter and Cole, 1943).

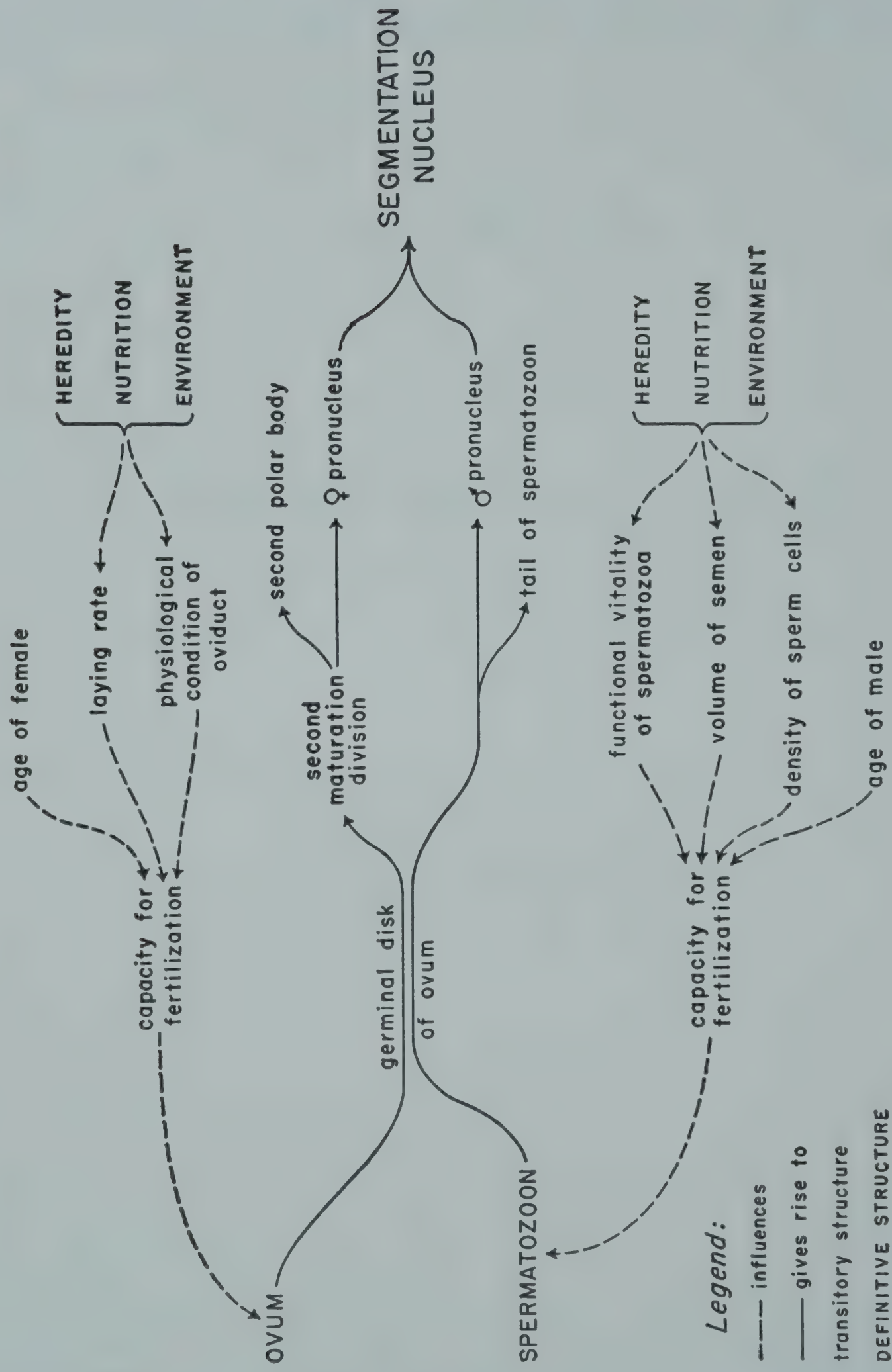


## CHAPTER TWO

# Fertilization and Fertility

*The act of gametes' conjugation  
(Excepting an infertile few),  
With strong genetic domination  
Creates some characters anew.*





## FERTILIZATION AND FERTILITY IN AVES



# FERTILIZATION AND FERTILITY

The life of the individual bird may rightly be considered to begin at the moment when fertilization occurs, that is, when the nuclear material of the spermatozoon combines with that of the ovum. This union takes place within the body of the female parent shortly after the ovum is released from the ovary. The particular spermatozoon that performs the vital function of fertilizing the egg is one of many millions which have passed through the entire oviduct and which are in close proximity to the ovum at the time of ovulation. It cannot be said whether this sperm cell is selected purely by chance or whether it competes successfully with the others because of some inherent superiority that gives it an advantage.

In spite of the enormous numbers of spermatozoa present in the reproductive tracts of female birds in the breeding condition, all eggs are not necessarily fertilized. Among the eggs of wild birds, those that escape fertilization comprise a small but probably rather constant percentage. The eggs of domesticated birds, however, are infertile with a highly variable frequency. Many influences are brought to bear upon the fertility of birds propagated under human control. These influences warrant consideration because of their broad biological implications.

## THE FERTILIZATION OF THE EGG

In birds, the phenomena of maturation, ovulation, and fertilization of the egg are intimately related and follow each other in rapid sequence. These processes have been seen best in the pigeon, *Columba livia* (Harper, 1904; Bartelmez, 1912; Durme, 1914), but they have also been studied in other birds, such as the swallow, *Hirundo rustica*, the sparrow, *Passer domesticus* (Durme, 1914), the hen, *Gallus gallus* (Olsen, 1942), and the turkey, *Meleagris gallopavo* (Olsen and Fraps, 1944). Observations have not been numerous because of the difficulty of obtaining eggs at exactly the desired stage of a very transitory period, but the descriptions available are in fundamental agreement.

### Ovulation

After the extrusion of the first polar body (see Chapter 1), the ovum is released from the follicular capsule. In the chicken, ovulation may occur at



any time from 7 to 74 minutes after the laying of the previous egg, the average interval being 32 minutes (*Phillips, 1936*). The wall of the pediculated follicle ruptures at the unattached pole, along the stigma, which is a pale, nonvascularized streak lying in the anteroposterior axis. The ovum bulges out and escapes instantaneously, apparently under considerable pressure. It emerges into the "ovarian pocket" (*Curtis, 1910*), the portion of the body cavity immediately adjacent to the ovary. According to Warren and Scott (*1935b*), the vitelline membrane is so weak at this time that the yolk accommodates its shape to that of the space into which it falls, but Bartelmez (*1912*) stated that the pigeon's (*Columba livia*) ovum regains its shape immediately after its escape.

Shortly before ovulation occurs, peristaltic waves begin to pass over the unattached proximal portion (infundibulum) of the oviduct. Almost inevitably, the moving infundibulum comes into contact with the unruptured follicle or with the newly ovulated yolk, either of which is alternately clasped and released by the open, fimbriated end of the tube. Within a short time, the ovum is captured. In the chicken (*Gallus gallus*), its engulfment is accomplished in 3 to 35 minutes after ovulation (*Warren and Scott, 1935b*).

### The Site of Fertilization

Penetration of the vitelline membrane by spermatozoa probably takes place in the infundibulum (*Patterson, 1910*). Harper (*1904*), however, found fertilized pigeons' (*Columba livia*) yolks in the body cavity and therefore concluded that fertilization occurs as soon as the germinal disc is exposed by the rupture of the follicular wall, the spermatozoa being borne in the liquid surrounding the ovum. Fertile hens' eggs have been obtained after the experimental introduction of semen into the ovarian pocket (*Kosin, 1944a; Drimmelen, 1945a; Olsen and Neher, 1948*), but it is possible, of course, that spermatozoa found their way into the infundibulum and entered the ovum there.

In the past, various scientists, including Buffon (*1771, Vol. II*), Burdach (*1838*), and Coste (*1850b*), expressed the opinion that fertilization takes place in the ovary. In more recent years, Ivanoff (*1924*) and Vermeulen (*1929*) reached the same conclusion. Ivanoff suggested that spermatozoa penetrate through the walls of mature and immature follicles alike, since irrigation of the oviduct with spermicides failed to prevent the appearance of fertilized eggs. Walton and Whetham (*1933*), after duplicating this experiment, nevertheless expressed doubt as to the ability of sperm cells to pierce the relatively thick membrane covering the immature ovum. Ivanoff's results can probably be explained by the ineffectiveness of spermicides against sperm cells lying in deep, inaccessible folds in the lining of the oviduct (*Drimmelen, 1946*). Tascher (*1876*) rejected the concept of



intraovarian fertilization after failing to find spermatozoa within ovarian follicles. Furthermore, experiments made by Olsen and Neher (1948) provide direct evidence that spermatozoa normally fertilize the ovum after ovulation. Follicles from inseminated pullets were removed and allowed to rupture *in vitro*. The ova thus obtained were transplanted to nonmated birds and were subsequently found to be infertile. Reciprocal transplantations from nonmated to inseminated birds yielded fertile eggs.

Other experiments indicate, nevertheless, that sperm cells may be able to penetrate the follicular wall and enter very young oöcytes (Olsen, 1952). After the direct application of semen to the surface of the ovary of 4- to 17-week-old chickens, structures strongly resembling male pronuclei appeared within immature oöcytes, and the division of many such oöcytes seemed to occur. A small percentage of apparently fertile eggs were laid 5 or 6 months after the date of operation.

### *The Transport of Spermatozoa through the Oviduct*

Because the fertilization of the avian egg takes place at the upper end of the oviduct, it is necessary that spermatozoa traverse the entire length of the tube. In birds, the oviduct is greatly elongated when it is in the active reproductive condition. In the laying hen, it is about 64.8 cm. long, and may even be as long as 82 cm. (Mimura, 1939). According to Romanoff and Romanoff (1949, p. 185), it consists of several regions, which, from cephalic to caudal end, on an average, are as follows: the infundibulum (7.0 cm.); the albumen-secreting portion (33.6 cm.); the isthmus (8.0 cm.); the uterus (8.3 cm.); and the vagina (7.9 cm.).

Experimental work, mainly with chickens, indicates that the progress of spermatozoa up the oviduct is extremely rapid. Estimates of the minimum time required for their passage vary from a few minutes (Martin and Anderson, 1918) to 10 hours (Tascher, 1876). Mimura (1939), using the technique of artificial insemination, recovered sperm cells from the infundibulum 26 minutes after placing them in the oviduct. Since this author introduced semen at an unnaturally high level, it is possible that somewhat more time is normally necessary; nevertheless, chicken spermatozoa can apparently complete their journey in less than an hour (Fronza, 1926). Ivanoff (1913) found spermatozoa throughout the length of the pheasant's (*Phasianus colchicus*) oviduct 5 hours after their introduction into the uterus.

It is unlikely that the motility of spermatozoa is solely responsible for their speed. Several other mechanisms are apparently called into play to assist in transporting the sperm cells through the oviduct.

If spermatozoa progress independently at any level in the female tract, it is probably in the uterus that they do so. Munro (1938d) observed that fowl sperm cells remained motile at the chicken's body temperature when



placed in the secretion of the hen's uterus. At the same temperature, they were immobilized in fluids from the albumen-secreting and infundibular regions of the oviduct, probably because of complex effects of temperature and the pH of the medium.

The property of rheotaxis said to be shown by sperm cells *in vitro* has been discounted by Parker (1931) as a laboratory artifact, but Makhovka (1939) has claimed that its existence can be demonstrated under properly controlled conditions. This property, if real, would assist migration through the vagina and uterus. The antiperistaltic activity of these portions of the oviduct has also been regarded as an important factor in the transport of spermatozoa (Parker, 1930, 1931).

Above the uterus, spermatozoa probably do not depend upon their own powers of locomotion. Mimura (1939) showed that fixed and stained spermatozoa and particles of powdered charcoal were carried through the oviduct with the same speed as living sperm cells. The means of conveyance may be the beating of cilia in the lining of the oviduct. Although it was once believed that all oviducal cilia beat toward the cloaca, Parker (1930, 1931) demonstrated the presence of cilia sweeping in the opposite direction in the genital tract of the female pigeon (*Columba livia*). The pro-ovarian cilia lie in a band occupying about one fourth of the circumference of the tube from the isthmus to the infundibulum. It is quite possible, also, that antiperistalsis supplements the influence of ciliary current (Mimura, 1939).

In the chicken flagellated spermatozoa are not ordinarily demonstrable in the lower regions of the oviduct more than 48 hours after natural mating (Drimmelen, 1945a), although Payne (1914) was able to find them throughout the length of the tract 7 days afterward. It has been said that many sperm cells lose their tails within 6 hours of entering the oviduct, and that all have lost them within 24 hours (Warren and Kilpatrick, 1929). Drimmelen (1945a), however, found active and morphologically normal spermatozoa in the infundibulum 14 days after their artificial introduction into the vagina. Degenerating and probably nonfunctional spermatozoa have been identified in the oviduct 56 days after insemination (Payne, 1914).

Spermatozoa, after completing their migration up the oviduct, apparently collect in the infundibulum and penetrate into deep crevices in its lining. Tascher (1876) demonstrated their presence in small pits along the infundibular margin. Eleven cells were the most that he found in a single pit. Drimmelen (1946) has counted as many as fifty to eighty spermatozoa in "sperm nests" located more posteriorly in the infundibulum, close to the upper end of the albumen-secreting region. The cells lie parallel in crypts in the lining, with their heads in the fundi and their tails extending into the ducts of the crypts. They are so well protected that irrigation of the peritoneal cavity and the entire oviduct with spermicidal solutions may fail

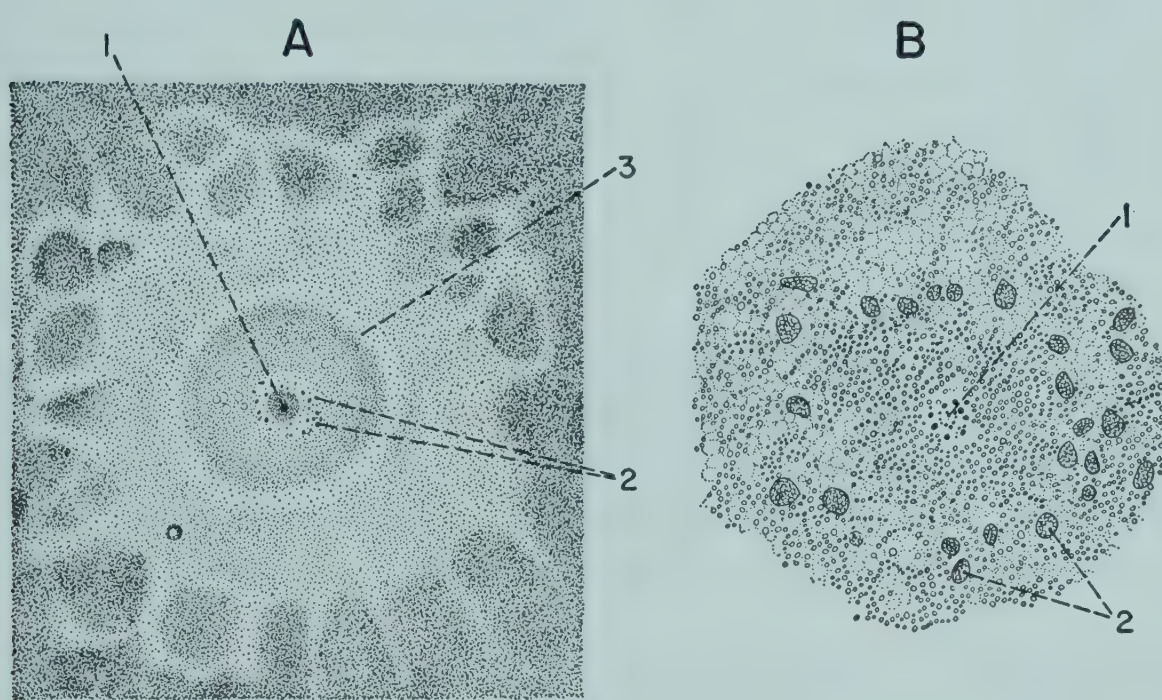


to destroy them (Ivanoff, 1924; Walton and Whetham, 1933). Certain nonciliated cells in the oviducal epithelium conceivably aid in maintaining the viability of spermatozoa (Drimmelen, 1949).

Kushner (1949) has stated that spermatozoa may penetrate into the cells of the oviducal epithelium and muscle in the hen.

### The Entrance of Spermatozoa into the Ovum

In the chicken (Olsen, 1942, 1952) and the turkey, *Meleagris gallopavo* (Olsen and Fraps, 1944), about 15 minutes normally elapses between ovulation and the entrance of spermatozoa into the ovum. Although only one spermatozoon is destined to fertilize the egg, several may succeed in passing



**Fig. 31.** Sperm nuclei in the immediate vicinity of the egg nucleus shortly after the entrance of spermatozoa into the blastodisc of the pigeon's (*Columba livia*) egg. (Redrawn with modifications after Harper, 1904).

A, section tangential to the surface of the ovum ( $\times 85$ ); B, the same ( $\times 700$ ).

1, chromosomes of egg in equatorial plate; 2, sperm nucleus; 3, polar ring.

through the vitelline membrane. Harper (1904) noted as many as twenty-five in a pigeon's (*Columba livia*) egg. Neither Patterson (1910) nor Olsen (1942), however, found more than five supernumerary sperm cells in chicken eggs, and Durme (1914) remarked that the presence of accessory spermatozoa is far from universal.

The sperm cells enter directly into the germinal disc. Figure 31-A is a surface view of the central portion of the germinal disc of a pigeon's (*Columba livia*) egg shortly after the entrance of spermatozoa. The supernumerary sperm cells may be seen in a narrow hyaline zone surrounding a granular area in which lie the chromosomes of the egg, in equatorial plate. Figure 31-B is a greatly enlarged view of the central portion of Fig. 31-A (cf. Fig. 13-E, Chapter 1).



### The Nuclear Phenomena of Fertilization

As if stimulated by the arrival of a spermatozoon in the germinal disc, the second maturation division of the egg nucleus now proceeds to completion. Bartelmez (1912) stated that the avian egg resembles other vertebrate eggs in reaching only the metaphase stage of the second polar spindle at the time of ovulation and progressing no further unless fertilization occurs. Durme (1914), however, claimed that spermatozoa do not enter the ovum until the second polar body has been formed.

In the second reduction division, the chromosomes are split longitudinally. The second polar body receives a complete set of chromosomes. In appearance and location, this polar body closely resembles the first, with the difference that it tends to enter a metabolic phase (Harper, 1904). Its mass of chromatin assumes a ragged outline as if about to form a reticulum (Fig. 32-A).

The egg nucleus now quickly reconstructs itself. A network develops from the chromatic material (representing the haploid number of chromosomes) that remains after the formation of the second polar body. A distinct nuclear membrane then becomes apparent, completing the reconstitution of the egg nucleus, which is often called the female pronucleus. In the ovum of the turkey (*Meleagris gallopavo*), the female pronucleus is spherical and about 10 microns in diameter (Olsen and Fraps, 1944); in that of the chicken, it is ovoid, measuring approximately 11 by 5.5 microns (Olsen, 1942).

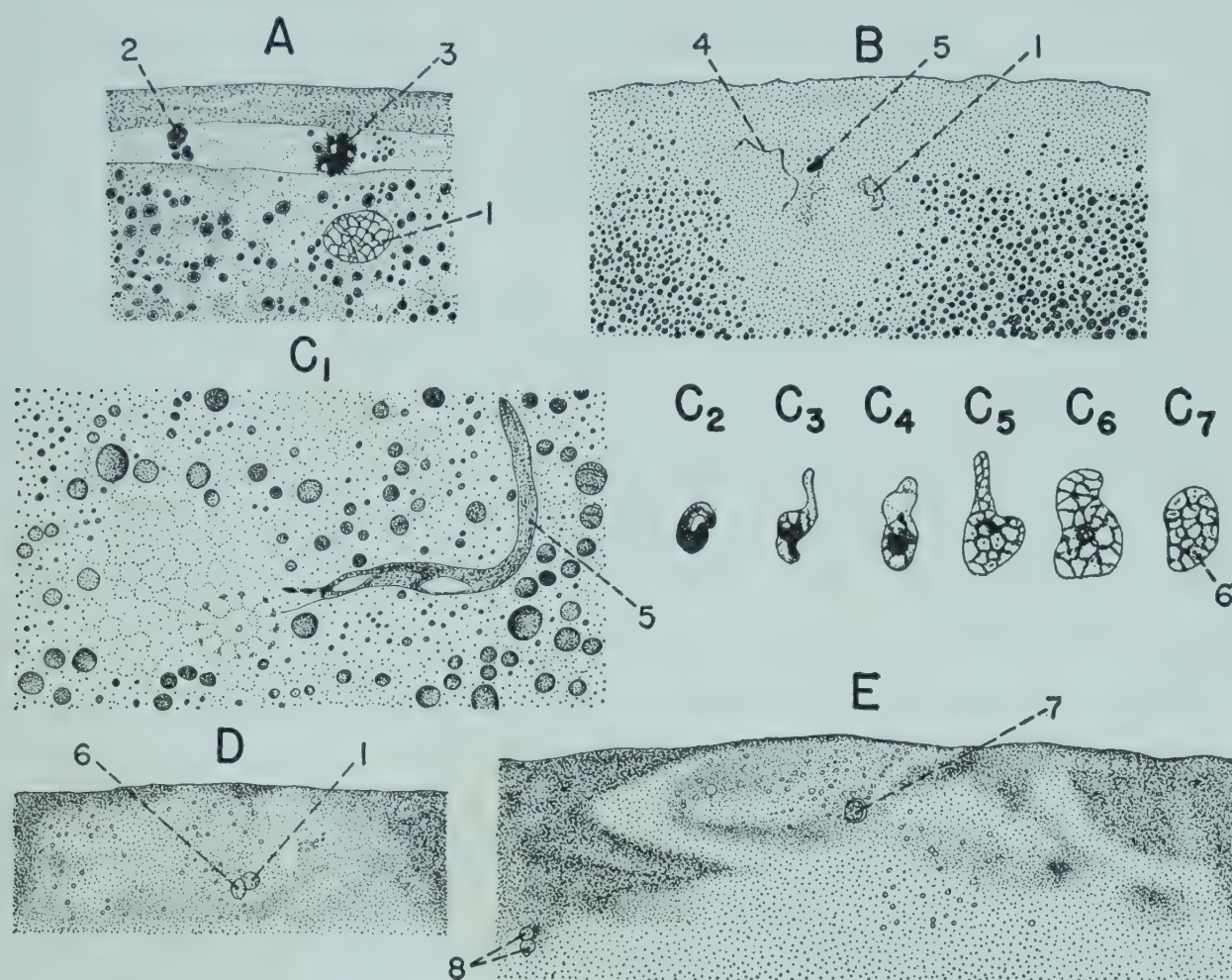
During this period transformations also occur in the spermatozoon. According to Durme (1914), the sperm cell buries itself in its entirety in the substance of the germinal disc (Fig. 32-B), and the tail then detaches itself. The head is gradually converted into a reticulated nucleus (Fig. 32-C<sub>1</sub> to C<sub>7</sub>), the male pronucleus, which is indistinguishable from the female pronucleus. If supernumerary spermatozoa are present, they undergo the same changes. The accessory sperm nuclei are sometimes found in the vicinity of the egg nucleus (cf. Fig. 31-B), but they migrate toward the periphery of the germinal area after the conjugation of the male and female pronuclei.

The actual fusion of the two pronuclei has not been seen. Harper (1904), however, observed the sperm and egg nuclei in contact, flattened against each other (Fig. 32-D). He also obtained a section containing the relatively large segmentation nucleus which results from the union of the parent nuclei (Fig. 32-E). The segmentation nucleus moves toward the surface of the ovum, and its chromatin becomes spun out into long threads in preparation for cell division.

Neher and Fraps (1946) found that the normal nuclear changes of maturation and fertilization occur in chicken eggs when ovulation is made to



occur prematurely through the injection of unfractionated anterior pituitary. These results indicate a relationship between ovulation and maturation and suggest that the latter process may be dependent upon the former, therefore ultimately under hormonal control.



**Fig. 32.** Successive events in the fertilization of the avian egg, from the extrusion of the second polar body to the formation of the segmentation nucleus. (Redrawn with modifications A, D, E, C<sub>1</sub>-C<sub>7</sub>, after Harper, 1904; B, after Durme, 1914.)

A, vertical section of a pigeon (*Columba livia*) blastodisc, showing first and second polar bodies and the reconstructed egg nucleus ( $\times 800$ ); B, vertical section through the blastodisc of a sparrow (*Passer domesticus*) egg, showing a spermatozoon close to the female pronucleus ( $\times 400$ ); C<sub>1</sub>-C<sub>7</sub>, successive stages in the transformation of a pigeon spermatozoon after entering an egg ( $\times 1800$ ); D, vertical section through a pigeon blastodisc, showing the male and female pronuclei in contact ( $\times 800$ ); E, vertical section of a pigeon blastodisc, showing the segmentation nucleus ( $\times 800$ ).

1, egg nucleus (or female pronucleus); 2, first polar body; 3, second polar body; 4, tail of spermatozoon; 5, head of spermatozoon in early stage of transformation; 6, sperm nucleus (or male pronucleus); 7, segmentation nucleus; 8, accessory sperm nuclei.

### Cytoplasmic Changes in the Blastodisc

From the time of the second maturation division, the cytoplasm of the blastodisc is in a state of flux. During the final reduction division, the blastodisc is almost doubled in size at the expense of the underlying white yolk (Durme, 1914). The second maturation spindle and the first polar body are found at the apex of a cone of granule-free substance extending to the periphery of the yolk (see Fig. 7-B, Chapter 1). The polar ring appears



in cross section as two V-shaped areas, one at either side of the cone-shaped mass (*Harper, 1904*). Numerous vacuoles are present throughout the active area. Beneath the blastodisc there is a lightly staining layer of yolk separating the disc from the deeper coarse yolk.

By the time the female pronucleus has formed, the cone-shaped area and the polar ring have been obliterated, apparently by cytoplasmic movements. When the pronuclei are in conjugation, cross sections show that there is a considerably vacuolated area at one side of the pronuclei and, at the other side, a hyaline channel (*Harper, 1904; Durme, 1914*) extending toward the surface of the ovum (cf. Fig. 32-D).

Harper (1904) stated that marked, amoeboid movements of the cytoplasm accompany the formation of the segmentation nucleus. In sections taken tangentially to the surface of the ovum, the cytoplasm around the segmentation nucleus is seen to be differentiated into a hyaline margin and a granular interior. This arrangement of the active portion of the cytoplasm apparently results from the spreading out of the cone of clear substance previously mentioned.

## FERTILITY

When applied to birds, the term fertility must be used in a rather narrow sense. It does not refer to the number of eggs or offspring produced, but to the proportion of eggs that are actually fertilized, whether or not all of them hatch when incubated.

The fertility of birds' eggs depends upon many conditions which may make themselves felt through either or both sexes. Some of these conditions affect all birds, whether wild or domesticated. Others have been discovered only through the study of domestic birds. Domestication, in imposing the influence of numerous factors that are of little importance in the wild state, demonstrates the variety of forces by which a biological phenomenon may be modified.

### Fertility in Wild Birds

Presumably, the fertility of wild birds' eggs is high. This statement, however, is based more upon what is known of the reproductive physiology of wild birds than upon data obtained from actual observation of their eggs.

Infertility among the eggs of the common tern (*Sterna hirundo*) is said to vary from 0.2 per cent (*Austin, 1929*) to 6 per cent (*Palmer, 1940*); among the eggs of the Arctic tern (*Sterna paradisea*), it approaches the latter figure (*Pettingill, 1939*). Over a period of 6 years, addled and infertile eggs together were found (*Nice, 1937*) to average 5.7 per cent of the eggs of the song sparrow (*Melospiza melodia*).



Studies of certain wild gallinaceous birds have yielded incidental information regarding the fertility of their eggs. The eggs of the ring-necked pheasant (*Phasianus colchicus*) may be about 96 per cent fertile (Twining, Hjersman, and MacGregor, 1948). They have been found to be 94 per cent fertile in an area where the ratio of cocks to hens was 1:7 (Randall, 1941), but normal fertility is probably maintained when the ratio is one cock to ten or twelve hens (Shick, 1947). A single mating may permit a pheasant hen to lay fertile eggs for 11 to 42 days (Shick, 1947), but the average duration of fertility is about 21 days (Twining, Hjersman, and MacGregor, 1948).

Among the eggs of the ruffed grouse (*Bonasa umbellus*), infertility may average from 2 or 3 per cent (Edminster, 1947, p. 288; Edminster and Crissey, 1947) to 5 per cent (Bump and Fordham, 1947). It is somewhat higher—from 4 to 5 per cent—in eggs of second clutches laid after the destruction of the first clutch (Edminster, 1947, p. 288; Edminster and Crissey, 1947).

When ruffed grouse (*Bonasa umbellus*) are kept in captivity, however, their eggs are at very best only 65 to 80 per cent fertile (Romanoff, Bump, and Holm, 1938; Bump, 1947; Bump and Fordham, 1947, p. 878). In contrast, the eggs of captive ring-necked pheasants (*Phasianus colchicus*) are ordinarily well over 90 per cent fertile (Romanoff, 1934, 1938; Romanoff, Bump, and Holm, 1938), and those of bobwhite quail (*Colinus virginianus*) in captivity are from 80 to 100 per cent fertile (Romanoff, 1934, 1938; Romanoff, Bump, and Holm, 1938; Bump, 1947). The fertility of pheasant (*Phasianus colchicus*) eggs may be as high as 87 per cent when one cock is confined in a pen with fifty hens (Twining, Hjersman, and MacGregor, 1948).

Some seasonal variation in fertility is apparent in the eggs of all three of the above species (kept in captivity). Infertility is lowest in April. At the beginning and near the end of the laying period, infertile eggs are of fairly common occurrence (Romanoff, Bump, and Holm, 1938).

### Fertility in Domestic Birds

The fertility of eggs is a matter of importance in the propagation of domestic birds and has therefore been the subject of considerable investigation. The large numbers of eggs laid by these birds has made it possible to gauge the extent to which fertility can be influenced by any one of an abundance of factors. Through the use of artificial insemination—originally an experimental procedure but gradually attaining greater practical importance—the study of fertility can be pursued under well-controlled conditions. Fundamentally, fertility appears to be an expression of physiological, ecological, hereditary, and even psychological conditions, operating through one sex or the other, or both.



### *The Direct Influence of Semen on Fertility*

The collective functional vitality of the sperm cells contained in a specimen of avian semen is of great significance in determining the proportion of eggs that will be fertilized by that specimen. Fertility, therefore, is to a large extent a measure of the fertilizing capacity of semen. Not all specimens of semen yield fertility of the same order, nor does the same specimen retain its maximum fertilizing capacity indefinitely, or equally well under all conditions.

The relative ability of avian semen to fertilize eggs is dependent upon various attributes of its cellular and noncellular components and may also be affected by a number of environmental forces.

**The Functional Maturation of Spermatozoa.** The fertilizing capacity of avian spermatozoa, like their motility, is gradually acquired as the cells pass through the male reproductive tract (*Munro, 1935*). The relative fertilizing ability (tested by artificial insemination) of sperm cells taken from the testes, epididymides, and vasa deferentia of the chicken is indicated in the accompanying table (*Munro, 1938c*). The spermatozoa of birds,

Source of Spermatozoa	Hens Laying Fertile Eggs (per cent)	Fertile Eggs Laid (per cent)
Testes	10.0	16.1
Epididymides	30.0	18.8
Vasa deferentia	93.8	65.3

therefore, resemble those of mammals in undergoing a period of maturation after formation, but maturity is apparently attained in the vasa deferentia rather than in the epididymides, which are very small organs in birds. Since the cells pass through the male tract with relative rapidity, aging does not seem to be a factor in the ripening process. The nature of the maturation process is not clear. Possibly protoplasmic changes occur within the spermatozoa, or perhaps a protective colloidal coating is added as the cells pass through the vasa. Although mature spermatozoa survive as long within the genital tract of the female as in that of the male, immature sperm cells do not ripen in the oviduct; evidently a male environment is necessary for the development of fertilizing capacity. Functional maturation perhaps depends upon the presence of the testis hormone (*Munro, 1938c*). Koch (1936) observed that cocks treated with testis hormone fertilized twice as great a percentage of eggs as untreated birds. He suggested that this effect is due to the action of a fraction of the testis hormone which exerts its influence within the sex gland, not upon the secondary sexual characteristics.

**Semen Volume and Sperm Cell Content.** Fertilizing effectiveness is correlated to some extent with the number of sperm cells and the volume



of suspending fluid with which insemination (natural or artificial) is performed.

The amount of semen ejaculated by a male bird at one time is very small, but the fluid contains an extremely large number of sperm cells. The average concentration of spermatozoa per cubic millimeter is considerably higher in chicken semen than in that of many mammals, including the rabbit (Munro, 1938a), the bull, the stallion, and the boar (Parker, McKenzie, and Kempster, 1942b). Spermatozoa are even more numerous per unit volume in turkey (*Meleagris gallopavo*) semen, which is ejaculated in much smaller quantity than chicken semen. It cannot be assumed, however, that comparable concentrations of sperm cells are present in the semen of all birds, for both Amantea (1925) and Owen (1941b) have found that the pigeon (*Columba livia*) falls far short of attaining the output of spermatozoa achieved by the chicken and the turkey.

In those species on which data are available, there is considerable variation in the total volume and sperm cell content of semen from different individuals and even from the same individual at different times. Table 2 shows how widely avian semen has been found to vary in these characteristics. As indicated, most of these data were obtained from studies of specimens ejaculated after abdominal massage, a technique for semen collection developed by Burrows and Quinn (1935, 1937). According to Parker, McKenzie, and Kempster (1942b), somewhat larger volumes of semen are collected by this method than by interception during natural mating.

Experiments with artificial insemination have shown that the fertilization of chicken eggs does not depend on sheer numbers of sperm cells (Hutt, 1929; Sampson and Warren, 1939; Shaffner and Andrews, 1948). Similarly, there is no direct correlation between fertilizing capacity and sperm cell concentration, provided the latter falls within the fairly wide normal limits (Parker, McKenzie, and Kempster, 1942b; Raimo, 1943c). There is a noticeable drop in fertility, however, after insemination (1) with semen diluted with "sperm serum" (the fluid portion of semen) to contain a total of fewer than 100,000,000 spermatozoa (Munro, 1938a); (2) with undiluted semen containing no more than 1,000,000 cells per cubic millimeter (Parker, McKenzie, and Kempster, 1942b); or (3) with less than 0.05 cc. of undiluted semen (Burrows and Quinn, 1938). Semen diluted with Ringer's solution to concentrations of 20 per cent and 10 per cent may give as good results as pure semen (Bonnier and Trulsson, 1939b). Semen diluted more than 1:10 with sperm serum shows a decrease in fertilizing capacity more or less proportional to the magnitude of the dilution (Weakley and Shaffner, 1952).

A turkey (*Meleagris gallopavo*) hen may be successfully inseminated with a total of about 43,000,000 spermatozoa. Insemination with amounts of undiluted semen varying from 0.005 to 0.1 cc. does not significantly af-



TABLE 2  
Characteristics of Semen of Various Species of Birds

Species	Volume (cc.)	Total Number of Sperm Cells (millions)	Sperm Cells per cu. mm. (millions)	Type of Ejaculation	Investigator
Chicken ( <i>Gallus gallus</i> )	0.10-0.70	500+ 3,360- 4,232	1.900- 5.500	Natural	Payne (1914); Rolf (1916)
				Natural	Amantea (1922)
			0.002- 4.000	Natural	Craft, McElroy, and Penquite (1926)
	0.10-3.60 0.50		0.018- 8.900	Natural	Hutt (1929)
			0.115- 0.438	Natural	Ishikawa (1930)
				Induced	Burrows and Quinn (1937)
			1.000-10.200	Induced	Munro (1938a)
				Induced	Burrows and Titus (1939)
			0.040- 1.800	Induced	Sampson and Warren (1939)
		0- 5,250	0.000-10.000	Natural	Parker, McKenzie, and Kempster (1942b)
	0.27-1.50	12-15,620	0.030-11.200	Induced	Parker, McKenzie, and Kempster (1942b)
				Induced	Raimo (1943a)
				Induced	Wheeler and Andrews (1943)
Turkey ( <i>Meleagris gallopavo</i> )	0.10-1.32	0-10,000	1.270- 3.496	Induced	Reis (1945)
	0.00-2.25		0.250-10.200	Induced	Gallein (1948)
	0.10-0.70		1.150- 1.680	Induced	Burrows and Marsden (1938)
	0.20-2.00		0.050- 6.000	Induced	Parker (1946b)
	0.10-0.70			Induced	Gallein (1948)
Pigeon ( <i>Columba livia</i> )	0.14-0.60	1,300- 5,400	3.600-12.700	Induced	Amantea (1925)
	0.10-0.80			Induced	Owen (1941b)
Guinea cock ( <i>Numida meleagris</i> )	0.01-0.02	200	0.370	Induced	Owen (1941a)
	0.02	5-6			



fect the fertility of turkey eggs (*Parker, 1946a*). The high sperm cell concentration of turkey semen (see Table 2) no doubt explains the effectiveness of so small a quantity as 0.005 cc.

Geese (*Anser anser*) may be satisfactorily inseminated with 0.05 cc. of semen (*Grecka, 1940*). A 1:3 dilution of semen with saline solution has been found to fertilize 62 per cent of pigeon (*Columba livia*) and ring-necked dove (*Streptopelia decaocto*) clutches (*Owen, 1941b*).

It appears that among chickens many attempted natural matings are not completed. Martin and Anderson (*1918*) observed a cock mate with thirty-three hens between 8:25 A.M. and 5:10 P.M. The fact that only ten hens laid fertile eggs afterwards suggests failure of the male bird to discharge semen in twenty-three of the thirty-three matings. There is good evidence that no semen is produced in 14 per cent (*Parker, McKenzie, and Kempster, 1942b*) to 32 per cent (*Penquite, Craft, and Thompson, 1930*) of all natural matings, with inevitable consequences on the fertility of the eggs laid.

*Factors affecting the volume and cell content of semen.* There are a number of factors that affect the volume of semen and the number of spermatozoa per unit volume.

One of the most important of these factors is the time interval between ejaculations (either natural or induced). Both the quantity of chicken semen and the concentration of sperm cells decrease as the rest period shortens (*Ishikawa, 1930*). The table shows the difference in the average

Time Interval between Samples (days)	Number of Spermatozoa per Ejaculate (billions)	
	Parker, McKenzie, and Kempster ( <i>1942b</i> )	Amantea ( <i>1922</i> )
0.125		0.07
0.25	1.0	
0.5	1.7	0.9
1.0	2.4	
1.5		3.6
2.0	3.0	4.2
3.0	3.8	3.0
8.0	6.1	

total number of cells per ejaculate in specimens obtained at intervals varying from 3 hours to 8 days. Successive specimens obtained at shorter intervals, such as every hour (*Penquite, Craft, and Thompson, 1930*), show the same tendency. The tabulated data indicate the quantitative changes in specimens of semen collected every 15 minutes (*Parker, McKenzie, and Kempster, 1942b*). After rest periods as long as 4 days, however, the volume of semen may be less than after a 2-day interval (*Burrows and Titus, 1939*).



Specimen	Volume (cc.)	Concentration of Spermatozoa (millions/cu. mm.)	Total Number of Spermatozoa (millions)
First	0.463	3.17	1,549.3
Second	0.291	1.96	731.1
Third	0.222	1.23	342.0

In view of the seasonal variation in the formation of spermatozoa in birds (see Chapter 1), it is not surprising that chickens produce larger amounts of semen during the spring months (*Penquite, Craft, and Thompson, 1930; Parker and McSpadden, 1943a; Wheeler and Andrews, 1943*), as may be

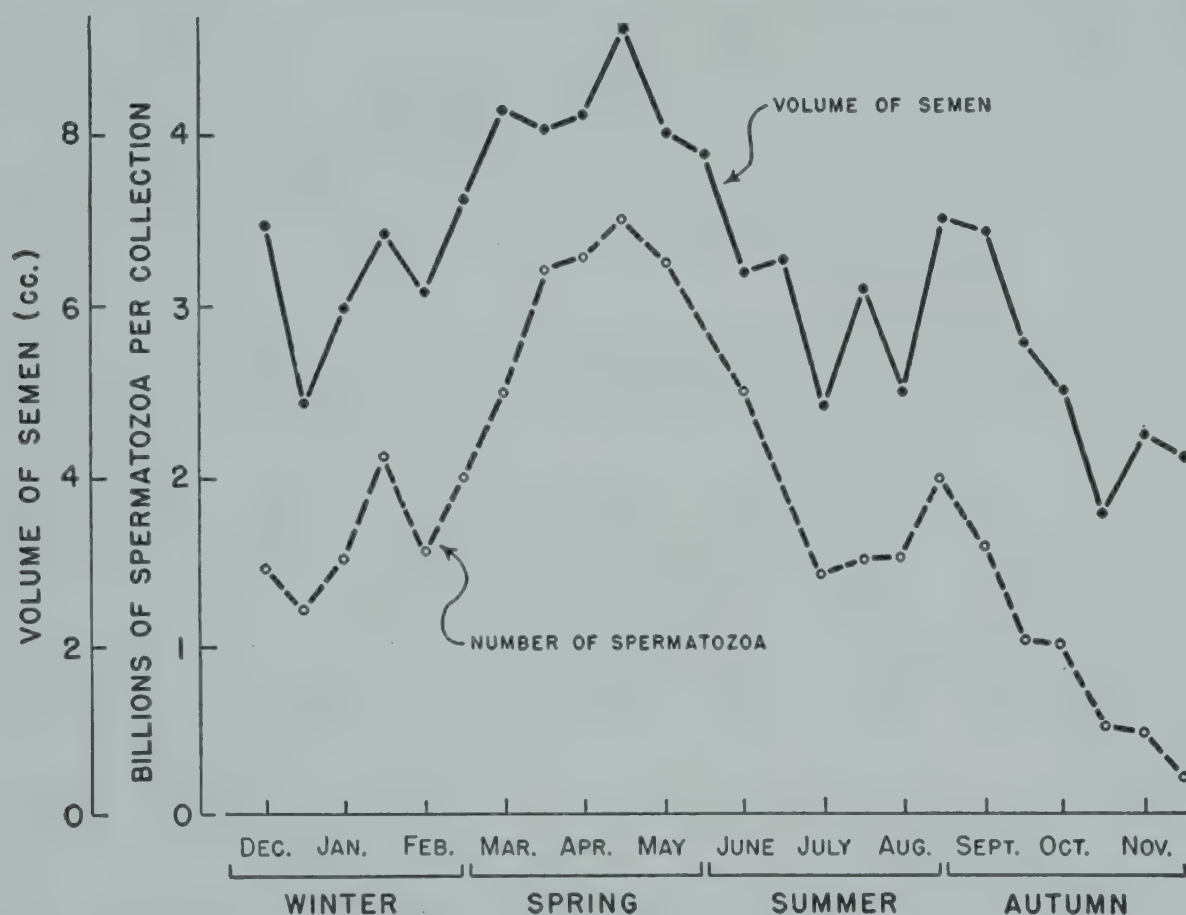


Fig. 33. Seasonal variation in the volume and total sperm cell content of semen produced by Rhode Island Red cockerels, *Gallus gallus*. (Plotted from the data of Parker and McSpadden, 1943b.)

seen in Fig. 33. An increased number of sperm cells per specimen of turkey (*Meleagris gallopavo*) semen has been found in March (*Parker, 1946b; Carson, Lorenz, and Asmundson, 1955*). The tabulated data indicate that the highest concentration of chicken spermatozoa also occurs in the spring (*Parker and McSpadden, 1943a*).

Month	Concentration of Spermatozoa (millions/cu. mm.)
January	2.0
March	2.7
May	3.8
July	2.8
September	2.2
November	1.0



Seasonal variations have been found in motility of spermatozoa (*Schindler, Volcani, and Weinstein, 1957*), and *Krzanowska (1956)* has observed fluctuations in sperm count, ejaculation volume, and rate of reduction of methylene blue of the semen. *Carson, Lorenz, and Asmundson (1955)* have observed that the number of sperm produced by turkeys (*Meleagris gallopavo*) varies with the time of year.

These seasonal changes result from the increasing length of the day during spring. Between 9 and 12 hours of daily illumination constitutes the threshold for increased semen production in the chicken (*Lamoreux,*

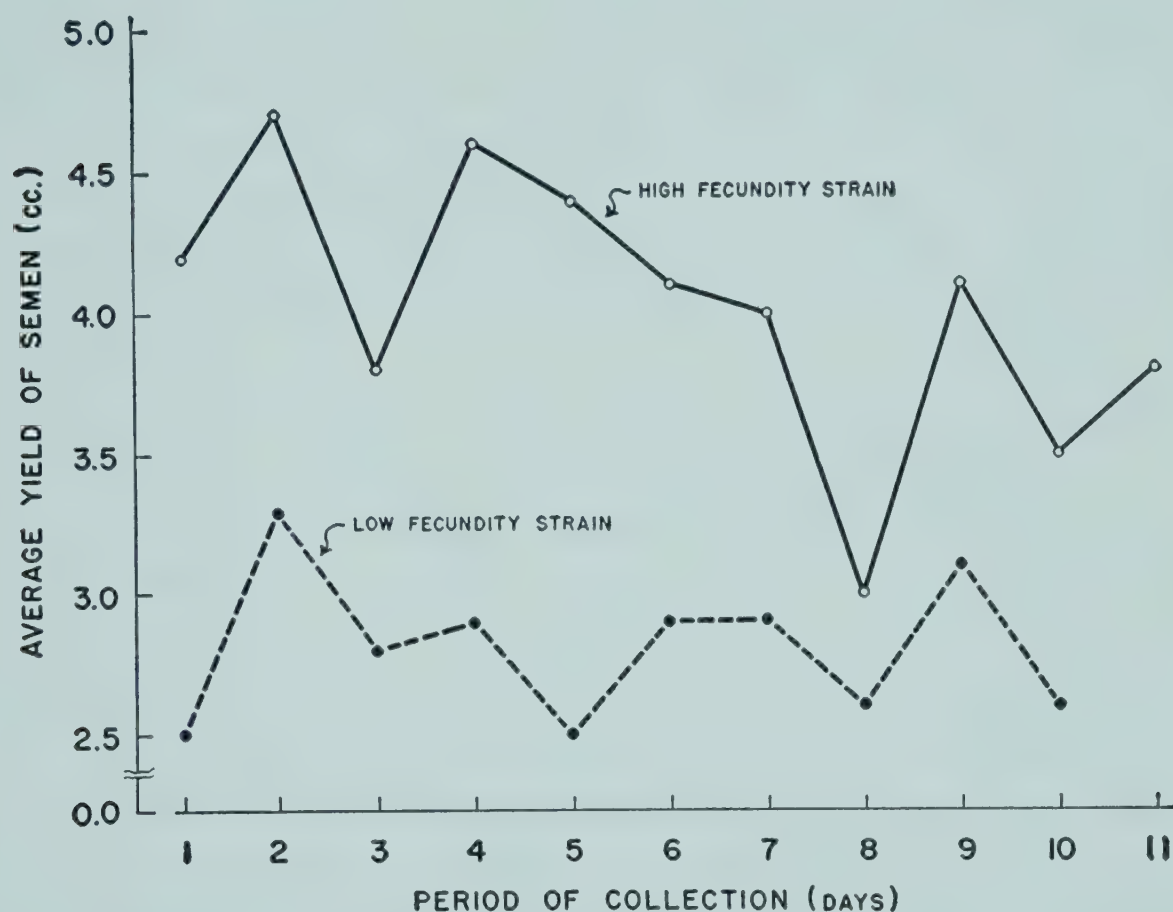


Fig. 34. Comparison of the average volume of semen collected daily during March from males of two strains of White Leghorn chickens (*Gallus gallus*), of which one strain was selected for high fecundity (egg production) and the other for low fecundity. (Drawn from the data of Jones and Lamoreux, 1942.)

1943). It has been shown that an artificial increase or decrease in average temperature has no effect on semen quality in chickens (*Wheeler and Andrews, 1943*); therefore the rise in mean temperature in the spring may be discounted as a factor.

The quantitative production of semen is possibly influenced by heredity. Jones and Lamoreux (1942) found that a consistently larger volume of semen was produced daily by males from a strain of White Leghorns (*Gallus gallus*) selected for high fecundity (as shown by egg production) than by birds from a strain selected for low fecundity. This superiority was evident in males of various ages from 12 weeks to 1 year. Figure 34 gives a comparison of the yield of semen obtained daily from the cocks of these two strains when the birds were approximately 1 year old.



Observations have been made of the variation in spermatozoa concentration in different species of chickens. Ferrand and Bohren (1948) noted a higher concentration of spermatozoa in the semen of Barred Plymouth Rock males than in that of New Hampshire cocks. White Leghorns have been found to produce semen of significantly greater volume and higher cell concentration than the semen of the native fowl of India (*Mukherjee and Bhattacharya, 1949*). Nevertheless, it cannot be stated that true breed differences exist, since strains within breeds of domestic fowl may vary considerably in many respects.

Young cockerels do not ejaculate semen in so large amounts as older birds. Burrows and Titus (1939) noted that the volume of semen obtained from cockerels increased until the birds were approximately 9 months old.

Feed restriction is thought to have an adverse effect on both the volume of semen and the number of spermatozoa produced by the chicken (*Parker and McSpadden, 1943b*). It is said that rations of deficient quality also lower the numerical concentration of sperm cells (*Craft, McElroy, and Penquite, 1926*).

The volume of semen produced by male chickens may be decreased as the result of feeding the birds desiccated thyroid or thyroprotein (*Titus and Burrows, 1940; Huston and Wheeler, 1949*), although there is a great variability in response to a given dosage (*Wilwerth, Martinez-Campos, and Reineke, 1954*). Much the same effect is produced by depression of thyroid function, achieved by the administration of thiouracil at the level of 0.5 per cent of the diet for 19 weeks (*Shaffner and Andrews, 1948*).

The injection of the cock with epinephrine (1:1000, 0.6 cc. daily per kilogram of body weight) greatly decreases semen volume and the total number of spermatozoa per ejaculate (*Wheeler, Searcy, and Andrews, 1942*). A 48 per cent reduction in volume and a 70 per cent decrease in the number of sperm cells has been found at the end of a 30-day injection period (Fig. 35).

Belenjkii (1940) reported that the average number of spermatozoa per cubic millimeter of semen was reduced by 33 to 45 per cent after injection of the cock with antitestis serum (prepared by injecting rabbits with extract of chicken testis).

Estrogens implanted subcutaneously in pellet form cause marked reduction of seminal volume, with no apparent effect on quality of the semen (*Akpınar and Shaffner, 1953, Eaton, Carson, and Beall, 1955*).

**The Motility of Spermatozoa.** Comparison of the semen of different male chickens indicates that there is a general and sometimes highly significant correlation between fertilizing capacity and the number of spermatozoa showing vigorous motility (*Parker, McKenzie, and Kempster, 1942b; Shaffner and Andrews, 1948*).

Motility, however, is not always a reliable criterion of the functional



condition of spermatozoa. At  $0^{\circ}\text{C}$ ., chicken sperm cells may retain their capacity for movement for several days (see Chapter 1), but they become completely nonfunctional after a few hours at this temperature. *In vitro*, they tend to lose their motility and their fertilizing power simultaneously and rapidly when held at temperatures between  $20^{\circ}\text{C}$ . and  $40^{\circ}\text{C}$ . (Garren and Shaffner, 1952). The immobility of spermatozoa at  $40^{\circ}\text{C}$ . in the fluids from the infundibular and albumen-secreting portions of the hen's oviduct indicates that they are nonmotile at upper levels of the female reproductive tract (Munro, 1938d), where they are nevertheless functional. In this case, immobilization no doubt prolongs their life by preventing the utilization of their stored energy (Munro, 1938d; Garren and Shaffner, 1952).

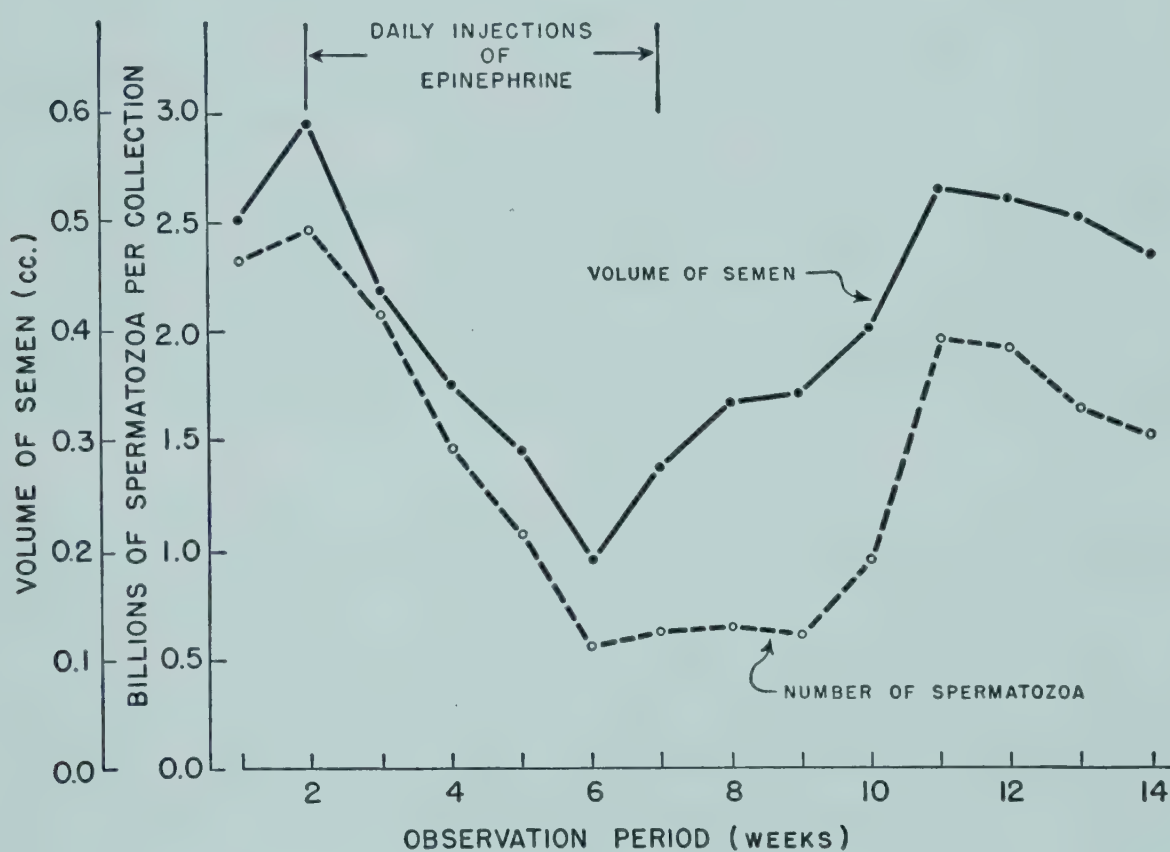


Fig. 35. The effect of intermuscular injections of epinephrine (1:1000, 0.6 cc. per kg. of body weight) on the volume and total sperm cell content of semen produced by male Plymouth Rock chickens, *Gallus gallus*. (Drawn from the data of Wheeler, Searcy, and Andrews, 1942.)

**The Morphology of Spermatozoa.** Morphological defects in spermatozoa are fairly common, even in the semen of male chickens with normal fertility records. The types of abnormal sperm cells most frequently encountered by Parker, McKenzie, and Kempster (1942b) are shown in Fig. 36, with the frequencies of their occurrence. Spermatozoa with coiled tails, broken tails, and missing tails were present in over 90 per cent of the samples examined, and 69 per cent of the specimens contained cells with coiled heads. Other investigators have also observed that tailless spermatozoa and cells with coiled tails or blunt heads constitute the majority of the defective forms.

A large proportion (over 20 per cent) of morphologically abnormal



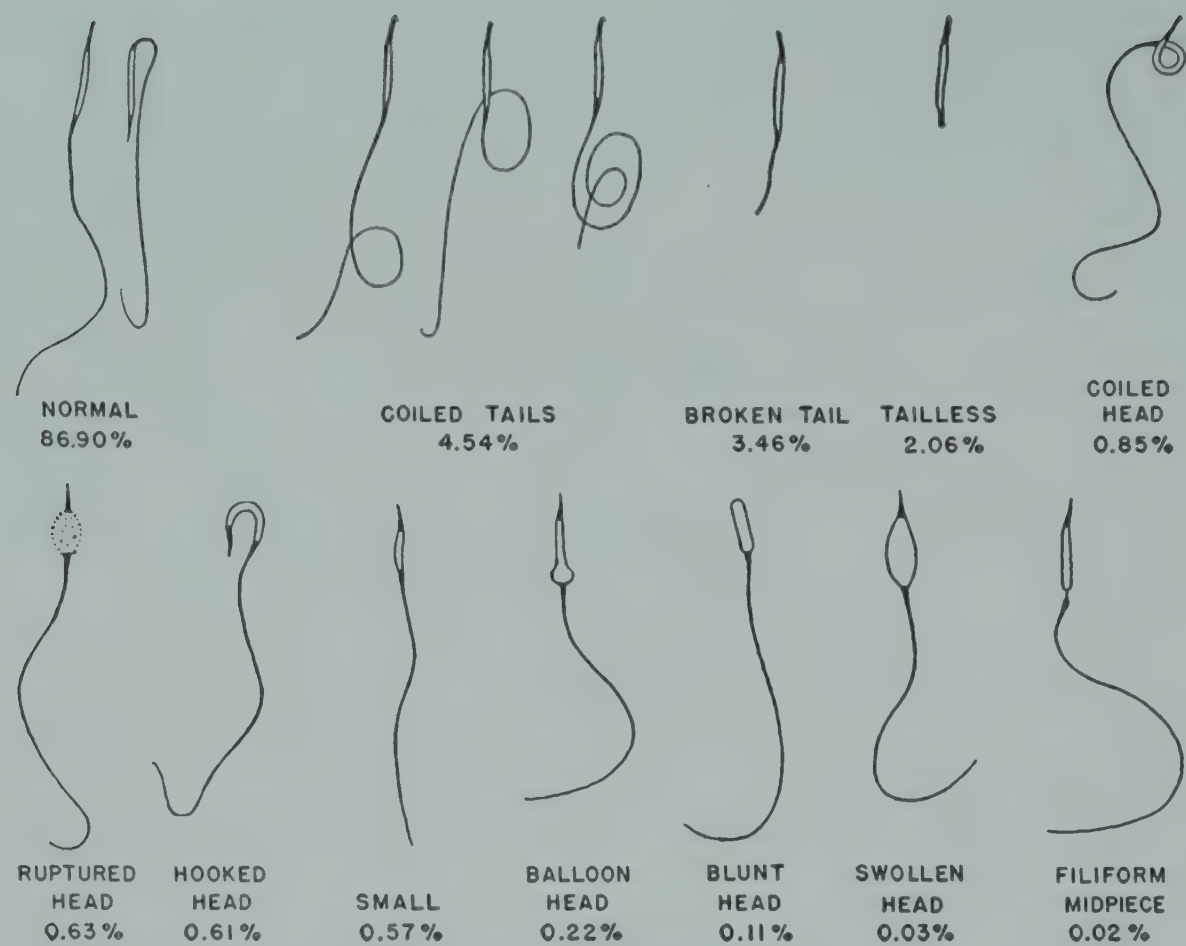


Fig. 36. Type and frequency of abnormal spermatozoa found in the semen of the male chicken, *Gallus gallus*. (Redrawn with modifications from Parker, McKenzie, and Kempster, 1942b.)

spermatozoa is incompatible with good fertilizing capacity (Sampson and Warren, 1939; Parker, McKenzie, and Kempster, 1942b), as shown by the tabulated data (Parker, McKenzie, and Kempster, 1942b). In the largest proportion (39 per cent) of the semen samples examined by Parker, McKenzie, and Kempster (1942b), there were 10 to 15 per cent abnormal cells; the maximum of 35 to 40 per cent abnormal cells was found in 1.4 per cent of the samples.

Abnormal Cells (per cent of total)	Eggs Fertilized (per cent)
0.0- 4.9	57.7
5.0- 9.9	73.8
10.0-14.9	66.6
15.0-19.9	51.8
20.0-28.0	29.5

Chicken spermatozoa suffer morphological changes when held *in vitro*. After 12 hours' storage in the refrigerator, the head portions of many cells show a tendency to become curved or even curled into a circle, and the tails exhibit considerable contortion (Kosin, 1944a). Eventually, stored spermatozoa become cytolyzed.

Treatment with X-rays also produces morphological changes. Kosin (1944a) noted that sperm cells show a marked tendency to curl up into



tight circles within 18 hours after treatment with 13,000 r of soft X-rays and within 3 hours after the administration of 76,000 r to 152,000 r. Spermatozoa exposed to any of these dosages are not functional.

**The pH of Semen.** Chicken semen is ordinarily slightly alkaline, its average pH being approximately 7.5 (*Parker, McKenzie, and Kempster, 1942b*). However, it may range in pH from 5.3 (*Wheeler and Andrews, 1943*) or 6.1 (*Kosin, 1944a*) to 8.5 (*Parker, McKenzie, and Kempster, 1942b*). If specimens are collected from the same bird at short intervals, each successive ejaculate is likely to be somewhat more alkaline than the one preceding it (*Zagami, 1937; Parker, McKenzie, and Kempster, 1942b*). During storage *in vitro*, semen tends to show an increase in pH (*Zagami, 1937; Kosin, 1944a*).

According to *Parker, McKenzie, and Kempster (1942b)*, specimens of chicken semen ranging in pH between 7.40 and 7.64 are endowed with the best fertilizing capacity.

**Inherent Variability in Fertilizing Capacity.** Semen from different individuals may show inherent inequalities in fertilizing power. Specimens of semen from two male chickens may appear to be entirely comparable by all criteria, yet the spermatozoa of one bird will partially or completely suppress those of the other when placed in competition under identical conditions (*Curtis and Lambert, 1929; Bonnier and Trulsson, 1939a; Parker, McKenzie, and Kempster, 1942b*). According to *Drimmelen (1951)*, the poorest component of a mixed sample of semen determines the quality of the sample.

It is possible that close familial or breed relationship with the ova to be fertilized may act to the advantage of spermatozoa (*Dunn, 1927; Bonnier and Trulsson, 1939a; Parker, McKenzie, and Kempster, 1942b*), but there is no truly conclusive evidence to this effect. The existence of breed differences in the fertilizing capacity of chicken spermatozoa is perhaps indicated by experiments in which semen from New Hampshire males, when placed in the oviducts of New Hampshire females, proved superior in competitive ability to that of Barred Plymouth Rocks, which in turn was superior to that of White Plymouth Rocks (*Ferrand and Bohren, 1948*). White Leghorn spermatozoa seem to be at a disadvantage when competing with the sperm cells of other breeds in the oviducts of either Rhode Island Red hens (*Bonnier and Trulsson, 1939a*) or New Hampshire hens (*Parker, McKenzie, and Kempster, 1942b*). However, *Allen and Champion (1955)* have shown that samples of semen from White Wyandotte, White Leghorn, and New Hampshire males, mixed in equal proportions, sired approximately equal numbers of chicks of any one paternal breed in either White Leghorn or New Hampshire hens. Breed differences cannot be assumed in chickens until familial influences have been eliminated.

Selectivity in fertilization may depend to some extent upon factors resid-



ing in the female (Bonnier and Trulsson, 1939a), although it has not been established what these factors may be.

**The Longevity of Spermatozoa in the Oviduct.** The spermatozoa of domesticated birds retain their fertilizing capacity for a relatively long period of time after entering the oviduct, although chicken sperm cells by no means remain alive for an entire year, as Fabricius ab Aquapendente (1621) believed. Spermatozoa exhibit species differences in longevity (Fig. 37). Riddle and Behre (1921) found that ring-necked doves (hybrids of *Streptopelia decaocto*) laid fertile eggs no later than 8 days after separation

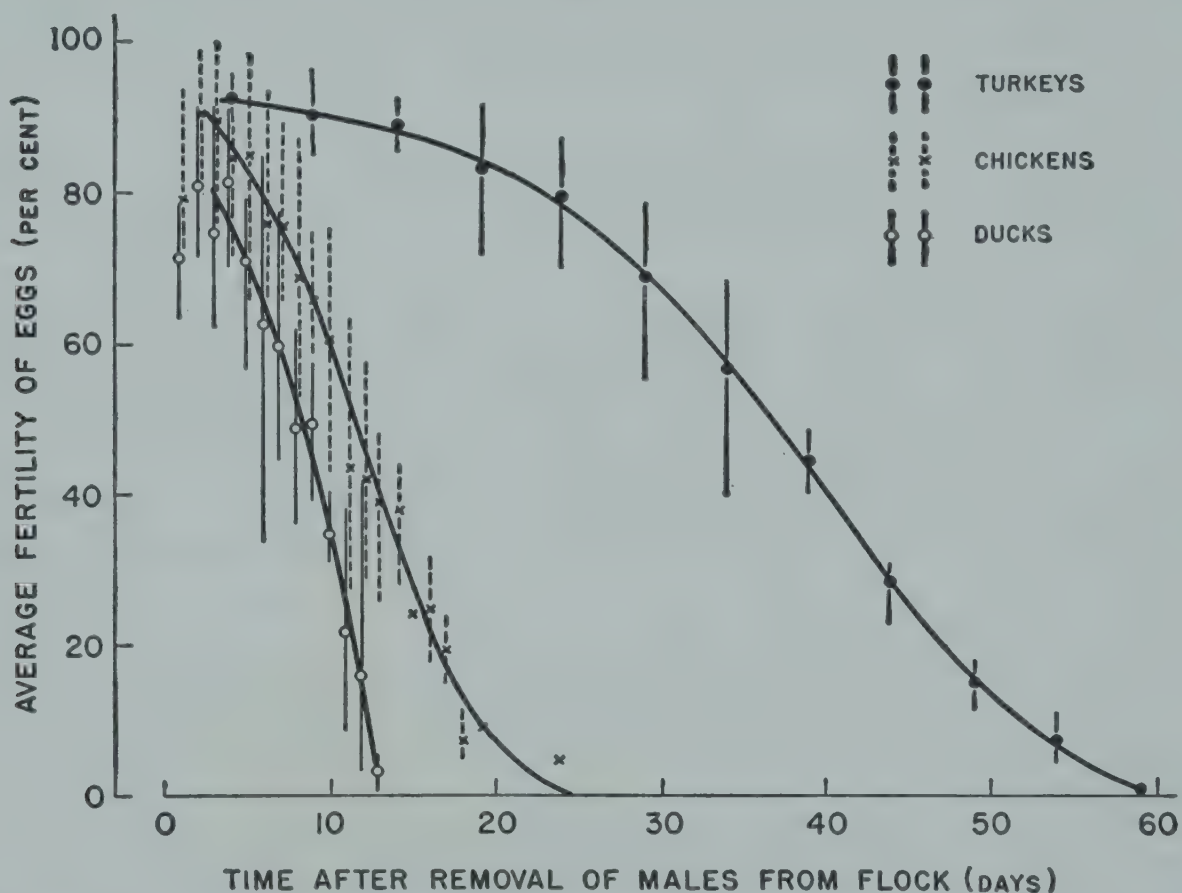


Fig. 37. The decline in the average fertility of the eggs of three species of domestic birds (ducks, *Anas platyrhynchos*, chickens, *Gallus gallus*, and turkeys, *Meleagris gallopavo*) following either a single insemination or the removal of males from the flock. (Curve for duck eggs constructed from the data of Fronda, Zialcita, and Dalisay, 1940; for chicken eggs, from the data of Waite, 1911b; Laurie, 1919; Ivanoff, 1924; Walton and Whetham, 1933; Parker, McKenzie, and Kempster, 1942b, and others; for turkey eggs, from the data of Scott, 1937, and Lorenz, 1950.)

from their mates, and Owen (1941b) observed that artificial insemination of pigeons (*Columba livia*) was effective for the same period of time. The mean duration of fertility for the goose (*Anser anser*), as noted by Johnson (1954), is 9.7 days, with a range from 4 to 16 days. By contrast, fertility in the turkey (*Meleagris gallopavo*) hen often persists for 30 days after mating (Scott, 1937; Burrows and Marsden, 1938) and has been known to endure for 55 to 59 days (Marsden and Martin, 1949, p. 182; Kosin and Wakely, 1950) and even for 72 days (Lorenz, 1950). Spermatozoa apparently perish within 11 to 13 days in the mallard (*Anas platyrhynchos*) or Muscovy duck's (*Cairina moschata*) oviduct (Chappellier, 1914, 1917; Fronda,



*Zialcita, and Dalisay, 1940; Tolentino, 1948*), or considerably sooner than in the hen's (*Gallus gallus*), where they may survive for 34 days (*Nalbandov and Card, 1943*). As the table shows, however, extremely variable reports have been given during the last 150 years on the maximum duration of the functional life of chicken spermatozoa in the hen's oviduct.

## Duration of Fertility

(days)	Investigator
7	Laurie (1919)
8	His (1868, <i>p.</i> 12)
14	Frature (1911); Rolf (1916); Penquite, Craft, and Thompson (1930); Drimmelen (1945 <i>b</i> )
15	Philips (1918); Walton and Whetham (1933)
16	Payne (1914); Kaupp (1919)
17	Coste (1850 <i>c</i> ); Laurie (1912); Fronda (1926)
18	Barfurth (1896); Sherwood and Buss (1913); Chappellier (1914, 1917); Gray (1916); Martin and Anderson (1918)
19	Lau (1894); Chlebaroff (1930)
20	Spallanzani (1785); Waite (1911 <i>b</i> ); Curtis (1928)
21	Ivanoff (1924); Curtis and Lambert (1929)
25	Parker, McKenzie, and Kempster (1942 <i>b</i> )
29	Lienhart (1922); Nicolaides (1934)
30	Lienhart (1923); Dunn (1927)
32	Crew (1926)
34	Nalbandov and Card (1943)

How variable is the survival time of chicken spermatozoa in the female tract becomes even more evident when it is noted that individual hens may cease laying fertilized eggs as early as the second day (*Frona, 1926; Curtis, 1928*) or the fourth day (*Nicolaides, 1934*) after insemination. Usually the percentage of fertile eggs laid by a flock starts to decline at some time between the fifth or sixth day (*Laurie, 1912; Gray, 1916; Nalbandov and Card, 1943*) and the fourteenth day (*Crew, 1926; Dunn, 1927*) after the removal of the male.

The loss of the senescent sperm cell's functional effectiveness has been shown particularly well by experiments in which the male (*Gallus gallus*) of the flock was replaced by another bird. According to Crew (1926), the period of a cock's influence is reduced to about half the normal time if he is superseded by a second cock. Warren and Kilpatrick (1929) observed an even greater curtailment in the duration of a supplanted bird's fertilizing capacity. They found that eggs fertilized by the substituted male appeared within 3 to 5 days, few or no eggs fertilized by his predecessor being laid thereafter unless the second cock was also removed. The results of alternate artificial inseminations with semen from two cocks led Bonnier and Trulsson (1939*a*) to conclude that the latest insemination is ordinarily responsible for fertilization.

Hens inseminated by intraperitoneal injection of semen lay fertile eggs



for 2 to 25 days (*Drimmelen, 1945a*). This period of fertility is similar to the normal period obtained after insemination, natural or artificial, *per vaginam*.

Prolonged vitality of spermatozoa in the hen's oviduct is apparently correlated with a high degree of sperm cell motility and a low percentage of morphologically abnormal cells (*Raimo, 1943b*). To a certain extent, however, the life of spermatozoa seems to depend on oviducal influences also.

Although senescent sperm can complete the act of fertilization, incubation of chicken eggs obtained four weeks after the last mating results in a very high incidence of embryonic abnormalities, particularly of the nervous

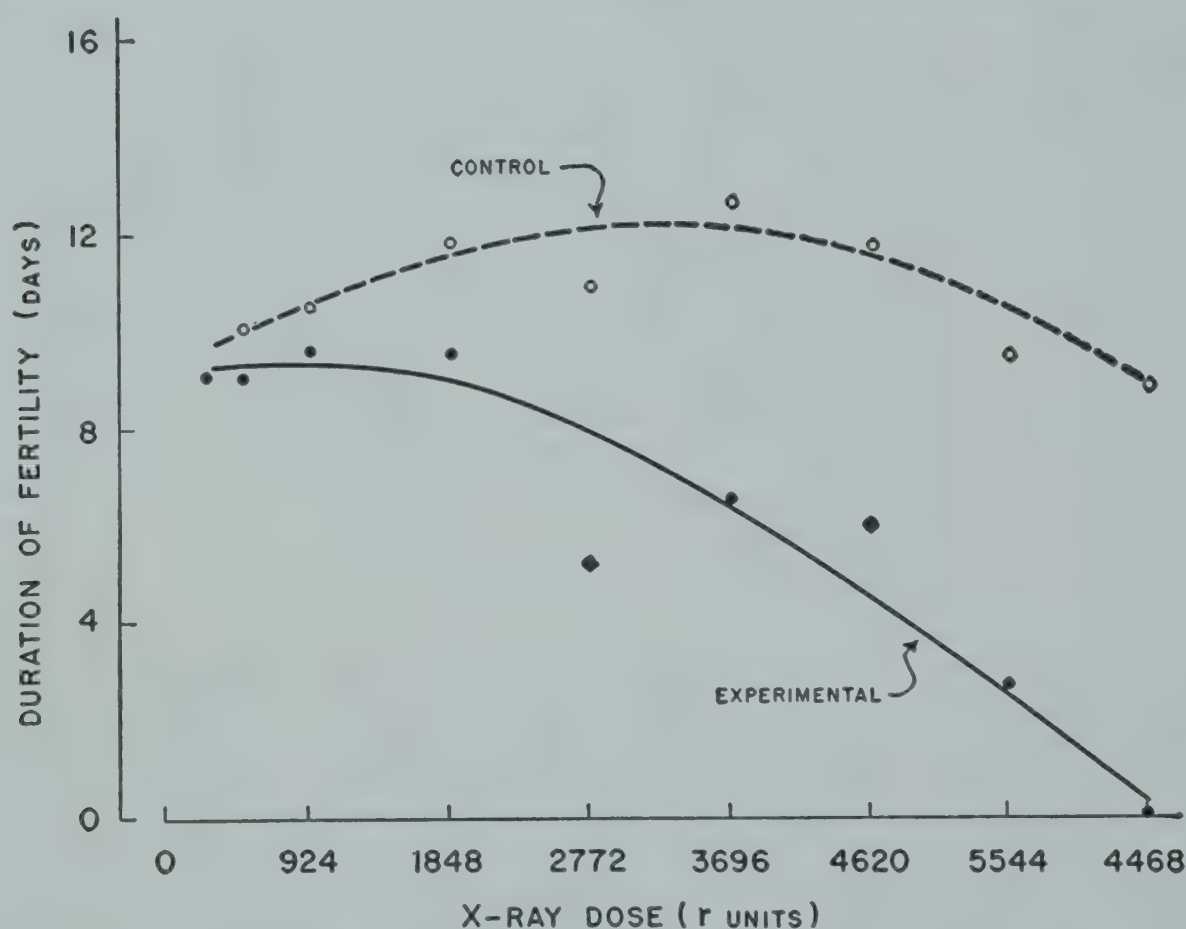


Fig. 38. The relationship between the intensity of the X-ray dosage administered to chicken (*Gallus gallus*) semen *in vitro* and the subsequent functional survival of spermatozoa in the oviduct of the hen (as shown by the duration of fertility following a single insemination). (Drawn from the data of Kosin, 1944a.)

and vascular systems, and development of these germs is retarded (*Dharmarajan, 1950*). Turkey (*Meleagris gallopavo*) hens, according to Olsen and Marsden (1953), produce similarly retarded and malformed embryos up to 224 days after isolation of the hen from the male.

**The effect of X-rays.** Irradiation of semen with X-rays shortens the life span of sperm cells in the oviduct. This effect is not particularly apparent within the first 10 days after insemination, unless the dosage exceeds 1848 r. As the intensity of irradiation increases above this level, there is a distinct reduction in the longevity of spermatozoa (Fig. 38).

**The Fertilizing Capacity of Semen Held *in Vitro*.** *In vitro*, the sperm cells of birds do not survive functionally as long as those of most mammals.

The search for procedures that will prolong the storage life of avian semen and thus facilitate the artificial insemination of domestic birds has led to the investigation of the effects of a number of environmental conditions.

*The duration of fertilizing capacity at various temperatures.* Undiluted chicken semen retains its fertilizing capacity for the longest period of time (at least 7 hours) when stored at 10° C. (Garren and Shaffner, 1952). A few eggs have been fertilized with semen held for 24 hours at 10° to 15.5° C. (Warren and Gish, 1943). At temperatures of 20° to 40° C., spermatozoa survive functionally for only 4 or 5 hours, probably because their metabolic rate is so elevated that their energy is quickly dissipated. At 0° C., they become nonfunctional in less than 3 hours, a phenomenon that is difficult to explain unless the cells are subject to "temperature shock" (Garren and Shaffner, 1952). However, chicken semen may be quick-frozen and held for 30 seconds at -6° C. without loss of the ability to fertilize (Shaffner, Henderson, and Card, 1941).

Some sperm cells retain their fertilizing capacity in semen partially dehydrated (by the addition of levulose) and held at -76° C. for an hour. Semen so treated may fertilize 25 per cent of eggs, but no embryos have survived beyond the tenth to fifteenth hour of incubation (Shaffner, 1942). On the other hand, at least half of all spermatozoa remain functional if semen which has been diluted with an equal volume of 30 per cent glycerol in Ringer's solution and quick-frozen at -79° C. is thawed at 40° C. and is then dialyzed against Ringer's solution at 20° C. for 2 hours to remove the glycerol slowly. Through the use of this method, it has been possible to obtain live chicks from about 70 per cent of eggs fertilized by semen held at -79° C. for 1 hour, and even from a few eggs fertilized by specimens held 33 days (Polge, 1951). (Twice the usual amount of semen must be used.)

*The effect of diluents.* The various solutions used to dilute semen prior to artificial insemination may have an adverse effect on the fertilizing capacity of spermatozoa. Thus Munro (1938a) found that the toxicity of Milovanov's solution for chicken spermatozoa is proportional to the degree of dilution, regardless of the density of the suspension. A 1:10 dilution of turkey (*Meleagris gallopavo*) semen with saline solution may reduce the fertility of eggs from 84 per cent to 40 per cent (Gilbreath and Davis, 1949), but normal saline solution is a satisfactory diluent for chicken semen (Weakley and Shaffner, 1952). On the other hand, fertility is lowered markedly when thin egg white is used as a diluent for chicken semen, even when the dilution ratio is only 1:3 (Weakley and Shaffner, 1952). Experiments indicate that the best diluent for cock semen is the fluid obtained by centrifuging a mixture of several specimens of semen, that is, the fluid in which spermatozoa are suspended naturally (Munro, 1938a; Weakley and Shaffner, 1952).



*The effect of X-rays.* Kosin (1944a) observed that soft X-ray irradiation of chicken semen reduces fertilizing capacity. Over a dosage range of 231 r to 5544 r (924 r per minute), there is an inverse linear relationship between the intensity of irradiation and the percentage of eggs fertilized after artificial insemination with treated specimens (Fig. 39). The administration of 6488 r completely destroys the functional power of sperm cells.

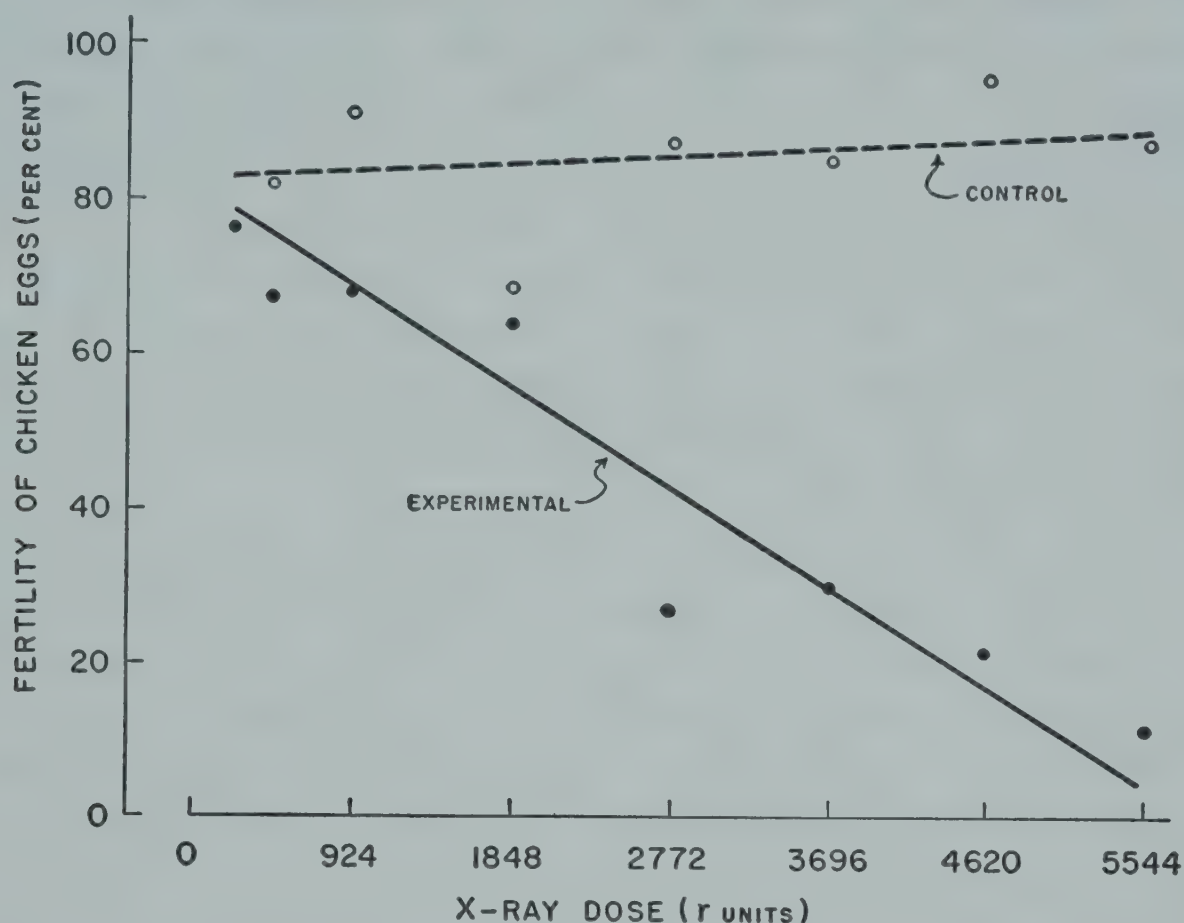


Fig. 39. The effect of various doses of soft X-rays on the fertilizing capacity of chicken (*Gallus gallus*) semen, as shown by the fertility of eggs laid following insemination with irradiated semen. (Drawn from the data of Kosin, 1944a.)

The adverse effect of X-rays is apparently due to a reduction in the number of living cells; after a sufficiently high dose, few if any spermatozoa remain alive or uninjured. Kosin (1944a) found that X-rays of less than the completely inactivating intensity did not alter the motility or morphology of spermatozoa or the pH of semen. Doses as high as 10,000 r had no effect on the oxygen quotient, succinic dehydrogenase system, or anaerobic glycolytic function of the cells. In the absence of detectable physiological disturbance, Kosin concluded that the proportion of eggs fertilized by an irradiated specimen of semen is determined by the numerical relationship between the functionally active and inactive cells contained in it.

Sturkie and co-workers (1949) have determined the correlation between X-ray dosage administered to the gonadal area of sexually immature male chickens and the fertilizing capacity of the same birds at 1 year of age. The tabulated data show the findings (Sturkie, Pino, Weatherwax, Donnelly, and Dorrance, 1949), which are compatible with observations on the effects of X-ray irradiation on spermatogenesis (see Chapter 1).

X-ray Dose (Roentgen units)	Average Fertility (per cent)
1200	70.7
1600	42.4
2100	20.0
2800	5.9
5600	0.0

*Physiological Influences Exerted through the Male*

Fertility may be influenced by certain factors acting through the male bird alone. These factors are physiological in nature and presumably affect the germ cells in some respect.

**The Age of the Male Bird.** The spermatozoa of the chicken do not acquire fertilizing capacity until the cockerel is at least 16 weeks old (*Hogue and Schnetzler, 1937; Parker, McKenzie, and Kempster, 1942a*), although fully formed cells may be present in the testes several weeks earlier (see Chapter 1). The average at which the male chicken becomes fertile is 24 weeks for the White Leghorn (*Parker, McKenzie, and Kempster, 1942a*) and 26 weeks for the Barred Plymouth Rock (*Hogue and Schnetzler, 1937*).

Irrespective of the age of his mates, the fertilizing ability of the cock declines as he grows older. The tabulation indicates the changes in the average percentage of eggs fertilized every year for 4 years by males mated each year to females of various ages, from less than 1 year to 3 years or more (*Hays and Sanborn, 1939*).

Age of Males (years)	Eggs Fertilized (per cent)
0.5–1.0	82.8
1.0	65.1
2.0	54.3
3 or 3+	27.2

**The Nutrition of the Male Bird.** Limitation of food consumption to 40 to 70 per cent of the amount normally taken is detrimental to the fertilizing capacity of the male chicken (*Parker and McSpadden, 1943b*). Less than one million spermatozoa per cubic millimeter may be produced by birds in advanced stages of inanition, a fact which probably accounts for poor fertility. In the spring, however, the reproductive system is more resistant to the influence of feed restriction than at other times. Inanition possibly affects the testes indirectly, through the hypophysis.

Kushner and Kondratink (*1946*) observed that superior fertilizing ability in male chickens of three different breeds was associated with a high content of hemoglobin in the blood, a characteristic usually indicating good nutrition.



There have been a few studies of the effect of carotenoid-free diets on the fertilizing capacity of the male fowl. In general, absence of carotene from the cock's ration has no effect on the fertilizing power of his semen (Schumacher, Scott, Hughes, and Peterson, 1944), unless the semen is placed in competition with that of birds on normal diets (Bohren, Carrick, and Andrews, 1945). Spermatozoa produced by cocks on low carotene diets may then be at a disadvantage; sometimes, however, it is not. A low carotene diet does not seem to affect the concentration, motility, or longevity (at 4° C.) of spermatozoa (Ferrand and Bohren, 1948).

Vitamin E is necessary for fertility in the male fowl, but the cock is capable of withstanding a long period of deficiency in this dietary element. Adamstone and Card (1934b) found that 2 years on a ration lacking in vitamin E were required to produce sterility in two cocks and that a third bird retained his fertilizing capacity. These investigators noted that an inadequate supply of vitamin E was eventually associated with a reduction in the number of osmophilic bodies in sperm heads. It was suggested, therefore, that the normal abundance of these bodies explains the high resistance of the rooster to a lack of the fat-soluble vitamin E, with which the testis of the fowl is probably richly supplied.

**The Endocrine Function of the Male Bird.** Depression of the thyroid gland may impair the functional efficiency of the cock's semen. The feeding of thiouracil as 0.5 per cent of the diet may cause fertility to decline from 94.7 per cent to 76.2 per cent, and also decreases the period of time over which an insemination is effective (Shaffner and Andrews, 1948). Similarly, thyroprotein may reduce average fertility from 91.5 per cent to 61.3 per cent when administered (as 0.02 per cent of the diet) to male chickens for 16 weeks (Shaffner, 1948). When given continuously from the hatching date, however, thyroprotein may have no effect on the fertility of natural inseminations, although it is possible that the frequency of mating may overcome any tendency toward reduced fertility (Huston and Wheeler, 1949.)

Pituitary hyperactivity has been postulated as the cause of the premature shedding of germ cells observed in the sterile males of a highly infertile strain of chickens (Gowe, 1949).

### *Factors Affecting Fertility through the Female Bird*

Factors affecting fertility solely through the female bird are not numerous. As might be expected, ovarian and oviducal influences are the most important.

**The Rate of Egg Production.** There is a positive correlation between the rate of egg-laying by chickens and the percentage of eggs that are fertile; that is, the birds that lay the most rapidly also produce the largest proportion of fertilized eggs. The number of eggs laid per week, for exam-



ple, provides a basis for demonstrating this relationship, as the tabulated data show (*Lamoreux, 1940a*). Since the birds that lay the most eggs also lay the largest number in succession, without pause, it follows that the fertility of eggs laid in long cycles is higher than that of eggs laid in short cycles (*Funk, 1939; Lamoreux, 1940a*).

Eggs Laid per Week (number)	Eggs Fertile (per cent)
1	68.8
2	74.7
3	80.4
4	82.5
5	86.1
6	87.1
7	88.2

Mating activity appears also to be correlated with the fertility of eggs and the rate at which they are produced. Heuser (*1916*) showed that the hens laying the largest number of eggs were those which mated most often. Lamoreux (*1940a*) attributed infrequent mating activity and poor fertility to the same cause as inferior egg production, namely, a low ovulation rate. However, he also found some evidence indicating that spermatozoa may survive somewhat longer in the reproductive tracts of high-producing hens.

It should be noted, on the other hand, that chickens with extremely high egg-laying records (300 eggs) during their pullet year may not maintain correspondingly high fertility subsequently. During their second, third, and fourth laying seasons, their fertility records may be inferior, in fact, to those of birds producing eggs at a considerably lower rate. It is possible that extraordinarily high egg production during the first year may be detrimental to future reproductive ability (*Hays, 1952a*).

Some intrinsic physiological relationship may exist between fertility and the rate of egg production (at the more usual levels, that is). Parker and McSpadden (*1943a*) observed that less than 2 per cent of eggs laid by yearling hens were fertilized as the result of artificial inseminations made in the fall, when birds of this age are laying very slowly, whereas 60 per cent of pullets' eggs were fertilized. Parker (*1947*) also noted a simultaneous decline in the fertility and egg production of turkey (*Meleagris gallopavo*) hens after April, regardless of the method of insemination (natural or artificial).

The great variation in the rate of egg production by different individuals also affects the latent period of infertility following insemination. The tabulation gives the time that a number of observers have found to elapse between insemination and the appearance of the first fertile chicken egg. It is obvious that the variable length of the infertile interval is due to the fact that some hens lay daily, others much less often or quite irregularly.



Time (days)	Investigator
2.00	Waite (1911a)
1.75	Sherwood and Buss (1913)
5.00– 6.00	Gray (1916)
1.00	Chappellier (1917); Laurie (1919)
1.20– 6.00	Martin and Anderson (1918)
0.96	Philips (1918)
0.63– 4.50	Lienhart (1922)
0.83– 1.00	Lienhart (1923)
1.60– 2.90	Crew (1926)
0.83– 9.90	Fronza (1926)
0.83– 5.00	Dunn (1927)
1.50– 3.00	Curtis (1928)
1.00– 3.50	Curtis and Lambert (1929)
2.00	Chlebaroff (1930)
0.81–11.90	Nicolaides (1934)
0.79– 3.40	Raimo (1943b)

**Oviducal Factors.** An egg descending the oviduct may offer a varying degree of obstruction to spermatozoa at different hours of the day. The time of day when mating occurs or when artificial insemination is performed therefore bears a relationship to the percentage of chicken eggs fertilized.

The best fertility results when insemination occurs shortly after egg-laying, regardless of the time of day, for the oviduct is then empty. Mimura (1939) showed that an egg in the uterus—where each egg spends approximately 20 hours (Warren and Scott, 1935b)—effectively prevents sperm cells from advancing for 12.5 hours. If a hard-shelled egg is present in the uterus at the time of artificial insemination, 30 per cent of the hens inseminated may fail to lay fertile eggs. Since the uterus most frequently contains a hard-shelled egg in the morning, this time is least favorable for insemination (Moore and Byerly, 1942; Malmstrom, 1943; Parker, 1945, 1950). On the other hand, inseminations accomplished in the afternoon, when there is often a membrane-covered or soft-shelled egg in the uterus, may result in a very high percentage of fertile eggs (Gracewski and Scott, 1943; Malmstrom, 1943; Parker, 1945). The latter finding has not been adequately explained as yet, although it is possible that conditions in the oviduct during the very early stages of eggshell formation provide a particularly favorable environment for spermatozoa (Parker, 1945).

The fertility of turkey (*Meleagris gallopavo*) eggs has not been found to be related to the hour when insemination occurs (Parker and Barton, 1946).

Oviducal factors also affect the time elapsing between mating or artificial insemination and the laying of the first fertile egg. Since the hen's egg traverses her oviduct in about 22 hours, a fertile egg may be laid on the day

following insemination (*Nicolaides, 1934*). The presence of an egg in the oviduct, however, may retard the progress of spermatozoa and thus delay the onset of fertility. The earliest appearance of fertilized chicken eggs was reported by *Lienhart (1922)*, who obtained them from pullets 15 and 17 hours after mating. It is difficult to explain this short interval, unless the passage of the egg is more rapid in pullets than in hens, or unless it is possible for fertilization to occur at a lower oviducal level than is commonly believed.

Like chicken eggs, turkey (*Meleagris gallopavo*) eggs also may be fertile on the day after insemination (*Lorenz, 1950*). Fertilized Muscovy duck (*Cairina moschata*) eggs are obtained after the lapse of not less than 24 hours from the time a drake is introduced into the flock (*Tolentino, 1948*). Since each egg of the pigeon's (*Columba livia*) two-egg clutch requires about 42 hours for formation (*Harper, 1904; Riddle, 1923*), artificial insemination of this bird must be performed at least 3 days before the first egg is laid; otherwise only the second egg is fertile (*Owen, 1941b*). Fertile first eggs have been obtained, however, only 48 hours after the intraperitoneal injection of semen (*Drimmelen, 1951*).

**Physiological Factors.** The advancing age of the female bird is the only physiological factor known to affect fertility. The investigation of a few other possible influences has yielded negative results.

**The age of the female bird.** The fertility of the female chicken, insofar as it is independent of the influence of the male, decreases as the bird grows older, but not so markedly as male fertility dissociated from the influence of the female. The data of *Hays and Sanborn (1939)*, given here-with, show that fertility decreases considerably from the pullet year to the third year when females are mated to yearling males, but decreases relatively little when females are mated to cockerels. *Jull (1935)* noted that White Leghorn females mated to cockerels were 6 per cent less fertile

Age (years)	Fertility When Mated to Cockerels	Fertility When Mated to Yearlings
	(per cent)	(per cent)
0.5-1	81.54	69.53
1	88.92	71.81
2	80.74	70.10
3 or 3+	80.15	48.87

when 2 years old than when 1 year old and 4 per cent less fertile as yearlings than as pullets. The same general trend has been recorded by other workers (*Hyre and Hall, 1932; Martin and Insko, 1934; Insko and Steele, 1944; Insko, Steele, and Wightman, 1947*). It is possible, however, that fertility resulting from a single mating may endure longer in hens than in pullets (*Curtis and Lambert, 1929*).



Asmundson and Lloyd (1935) found no significant decrease in the fertility of turkey (*Meleagris gallopavo*) hens up to the fifth year.

**Other factors.** Experiments on pullets indicate that the thyroid function of the female does not affect fertility (McCartney and Shaffner, 1950). Pituitary dysfunction leading to oviducal conditions that are unfavorable to spermatozoa has been proposed (but not investigated) as a possible cause of low fertility in the female chicken (Gowe, 1949).

Spermatozoal antibodies in the blood stream of the hen have been thought to cause infertility (McCartney, 1923). It has been found, however, that fertilization is not prevented when the formation of such antibodies is induced by the injection of semen (Lamoreux, 1940b).

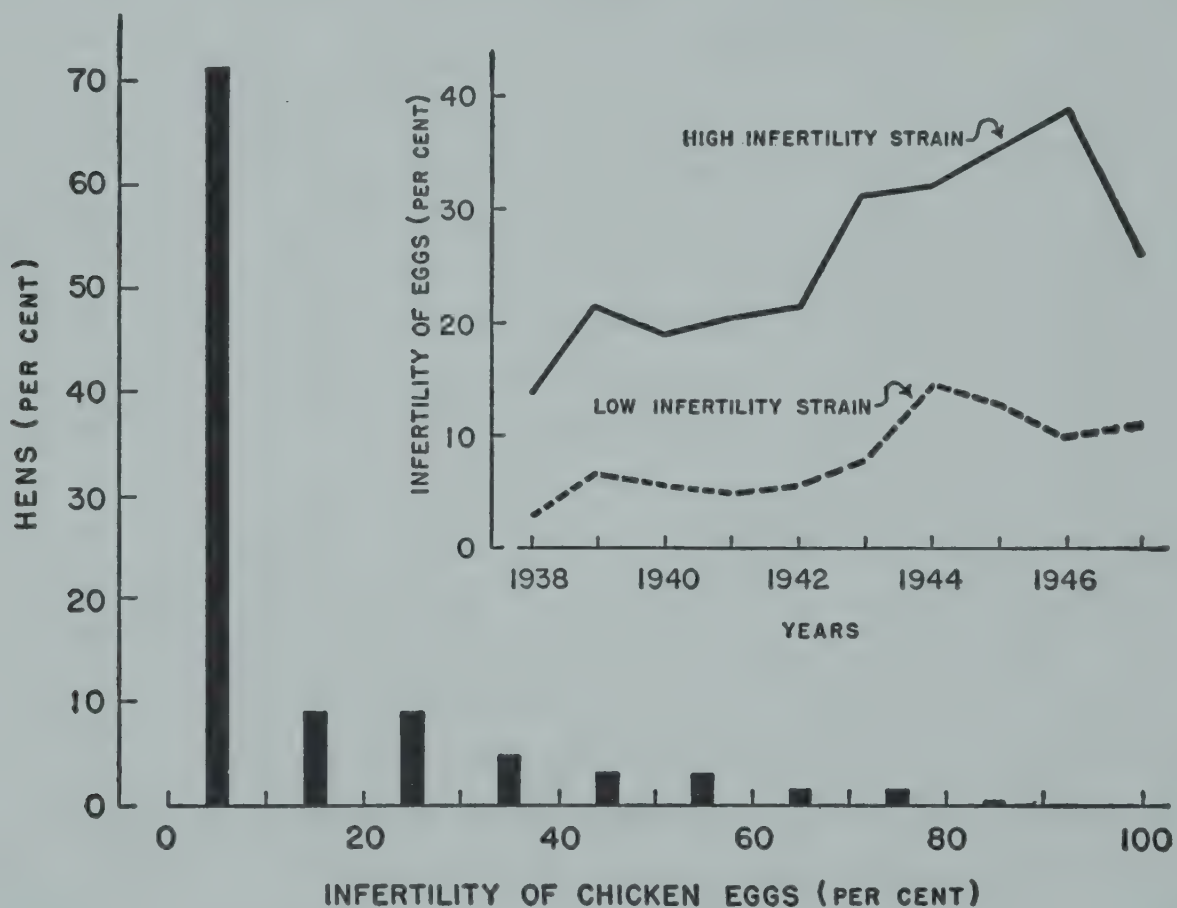


Fig. 40. The frequency distribution of 202 hens (*Gallus gallus*) according to the percentage of infertile eggs laid by them. (After Romanoff, 1944d.)

The insert at the right shows the persistence of high and low infertility in two strains of chickens. (Plotted from the data of Gowe, 1949.)

**The Inherent Fertility of the Individual.** The variation in the percentage of infertile eggs laid by the different individuals of a flock of hens (Fig. 40) may be due, of course, to the operation of a number of factors. One of these factors may well be the inherent fertility that seems to characterize each female chicken (Blyth, 1945). Pearl and Surface (1909) concluded that fertility in the female is independent of male fertility, although they failed to investigate various other influences. Furthermore, a varying proportion of hens (from 9 to 30 per cent) has been found to lay infertile eggs consistently (Hyre and Hall, 1932; Sampson and Warren, 1936; Bonnier and Trulsson, 1939b; Hays and Sanborn, 1939; Pyenson,



1939; Moore and Byerly, 1942; Parker, McKenzie, and Kempster, 1942b; Gowe, 1947, 1949). Occasional turkey (*Meleagris gallopavo*) hens exhibit the same unexplained infertility (Burrows and Marsden, 1938; Gilbreath and Davis, 1949).

The duration of fertility, too, seems to be an individual trait. According to Gowe (1949) each female lays fertile eggs for a fairly constant period of time regardless of the relative fertilizing capacity of the semen with which she is inseminated; however, Maw and McCartney (1956) have evidence from a study of inbred and outbred White Leghorns (*Gallus gallus*) which indicates that the genetic constitution of the cock is influential.

It is possible that the basis for partial or complete infertility or fertility of abnormally short duration is some physiological disturbance of a hereditary nature which affects the oviduct (Gowe, 1949).

### *Factors Affecting Fertility through Both Sexes*

There are certain factors affecting fertility which, by their very nature, make themselves felt through both sexes. These influences may be broadly classified as physiological, hereditary, psychological, and environmental.

**Physiological Factors.** The physiology of birds of both sexes, especially as affected by their diet and their age, inevitably is reflected in the fertility of the eggs produced.

**Nutrition.** The fertility of the eggs laid by a flock of hens cannot be maintained if all the birds, both male and female, do not receive sufficient vitamin D or ultraviolet light (Hart, Steenbock, Lepkovsky, Kletzien, Halpin, and Johnson, 1925). A normal percentage of fertilized eggs is assured if the flock is given cod liver oil (as 2 per cent of the diet, by weight) or is housed in quarters with windows of quartz-containing glass (Sheard and Higgins, 1929, 1930). Unless these precautions are taken, the percentage of fertilized eggs may decrease steadily, as Fig. 41 shows.

Both chickens and ducks (*Anas platyrhynchos*) also require adequate amounts of vitamin A to support normal fertility (Wagener and Harms, 1943).

It has been said that the fertility of chicken eggs is unaffected when both males and females are fed a diet free of carotenoid pigments (Palmer and Kempster, 1919). Some slight decline in the physiological efficiency of spermatozoa may result, however. It has been found, also, that access to green range improves fertility (Stephenson and Bryant, 1944).

A diet deficient in manganese may lead to a slight decrease in the fertility of chicken eggs (Gallup and Norris, 1939).

**The combined age of mated birds.** According to Pearl (1917b), who studied the effect of the combined ages of mates on the fertility of chicken eggs, there is a significant decrease in fertility on passing from a combined age of 2 years to one of 3 years, no change in passing from 3 to 4 years, and



a pronounced decrease in passing from 4 to 5 years. This statement expresses, in specific terms, the general decrease in fertility with age.

**Hereditary Influences.** It appears fairly definite that fertility is inherited in the chicken. Some workers (*Pearl and Surface, 1909; Hays and Sanborn, 1924, 1939*) have expressed doubt regarding the importance of heredity as a determining factor, but the great weight of evidence indicates that fertility is a familial trait. Gowe (1947, 1949), for example, made observations on two strains of White Leghorns (*Gallus gallus*), in one of which infertility averaged 28 per cent over an 11-year period as compared with 10 per cent in the other (cf. Fig. 40). Low fertility or complete infertility may be due to an inheritable endocrine dysfunction which is expressed in both sexes of the less fertile strain.

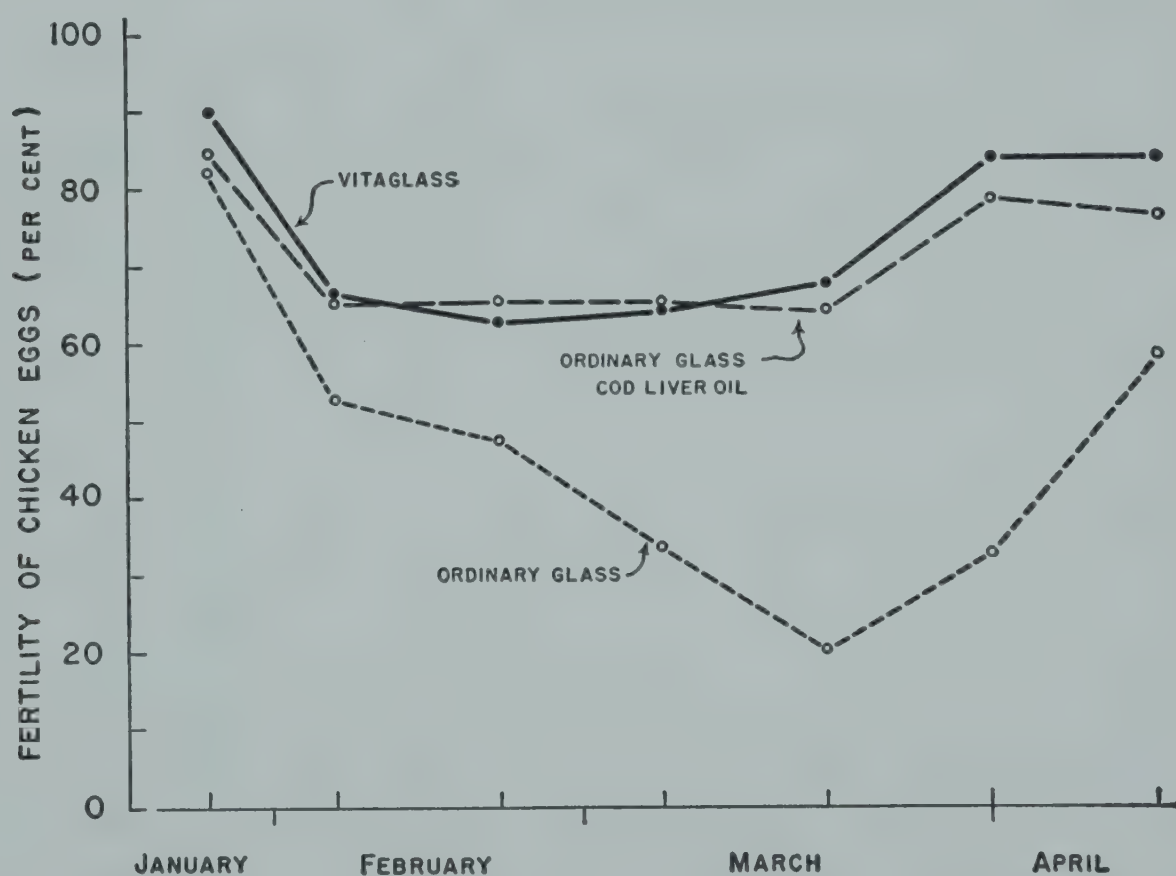


Fig. 41. The relationship between the fertility of chicken (*Gallus gallus*) eggs in winter and the amount of vitamin D or ultraviolet light received by the birds. (Plotted from the averaged data of Sheard and Higgins, 1930.)

The exact mechanism through which fertility may be inherited is not yet entirely clear. There are indications, however, that sex-linked genes for fertility are transmitted from sire to daughter (*Hays, 1950, 1952b*). There appears to be a correlation between fertility in the male and egg production in the female. Jones and Lamoreux (1942) studied male fertility in two strains of White Leghorns (*Gallus gallus*) selected for high and low egg production, respectively, and found a marked difference in favor of the birds from the high-fecundity strain. To some extent, this difference may have reflected the influence of the rate of laying on fertility, but the greater spermatogenic activity in the males of the high-fecundity strain (cf. Fig. 34) denotes a certain inherent superiority. High yield of semen appears to be

characteristic of inbred White Leghorn lines, indicating that it may be genetic (*Williams and McGibbon, 1956*). A correlation between the fertility of dams and daughters in Rhode Island Reds (*Gallus gallus*) has also been reported (*Jull, 1935*), but this finding has not been corroborated (*Hays, 1950*).

The effect of inbreeding on fertility in the chicken has not been fully determined. Although inbreeding has been said to cause a decrease in fertility (*Dunkerley, 1931; Jull, 1930, 1933*), it may not always do so (*Cole and Halpin, 1916; Wilson, W. O., 1948*), particularly if proper selection is practiced (*Knox, 1946*). *Waters and Lambert (1936)* observed that fertility declines only after several generations of inbreeding, if at all. Highly inbred strains, in fact, may be more fertile than random-bred strains (*Waters, 1938*). On the other hand, *Hays and Sanborn (1939)* found that the fertility of several types of inbred matings (matings of full or half-brother with sister, son with mother), although sometimes slightly superior to the average fertility of the flock, were distinctly inferior to the fertility which these authors obtained by outbreeding; matings of sire with daughter gave very poor results. In the opinion of *Bernier, Taylor, and Gunns (1951)*, the ill effects of inbreeding are most probably brought about indirectly by oviducal conditions inimical to spermatozoa.

Crossing two varieties of chicken may impair fertility somewhat in the progeny (*Knox, Quinn, and Godfrey, 1943*). The fertility of crossbred chickens may be lower than that of the more fertile parent and may even be lower than that of the less fertile parent (*Smith and Wiley, 1950; Godfrey, 1953*).

In chickens, lowered fertility may be associated with genes for certain mutations, such as rumplessness (*Dunn and Lanauer, 1934*) and "frizzling" of the plumage (*Landauer, 1942*). The low fertility of the rumpless fowl is probably the indirect result of morphological deformity which interferes with mating activity.

There are indications that fertility is inheritable in the turkey, *Meleagris gallopavo* (*Harper and Parker, 1950; Blow, Glazener, Dearstyne, and Bostian, 1951*). In this species, inbreeding appears to be detrimental to fertility to an extent more or less proportional to the degree of inbreeding (*Marsden and Knox, 1937*). Crossing two breeds of turkey has been said both to improve fertility (*Clark, Runnels, and Livesay, 1944; Walter and Hoffman, 1947*) and to impair it (*Marsden and Olsen, 1950*).

**Seasonal Influences.** Throughout the year, there is a consistent seasonal variation in the fertility of eggs laid both by chickens (*Upp and Thompson, 1927*) and turkeys, *Meleagris gallopavo* (*Marble and Margolf, 1936; Parker, 1947*). Fertility is highest in winter and early spring and decreases to its lowest level during the summer.

These fluctuations in fertility may result from more than one cause. No



doubt there is a correlation between fertility and the rate of spermatogenesis and oögenesis, both of which vary seasonally (see Chapter 1). Hays and Sanborn (1939) found that average fertility increased from 54 per cent on March 4 to 85 per cent on May 15. Although these workers believed that this increase paralleled an increase in mean temperature from  $-8.9^{\circ}\text{C}$ . ( $16^{\circ}\text{F}$ .) to  $7.2^{\circ}\text{C}$ . ( $45^{\circ}\text{F}$ .), it was more probably correlated with a seasonal increase in egg production. Lamoreux (1942) was unable to detect any relationship between fertility and temperatures within the range of  $5^{\circ}\text{C}$ . ( $23^{\circ}\text{F}$ .) to  $10^{\circ}\text{C}$ . ( $50^{\circ}\text{F}$ .) when the rate of laying was constant. The extremes of winter and summer temperature, however, appear to affect fertility adversely. When the temperature is in the neighborhood of  $38^{\circ}\text{C}$ . ( $100^{\circ}\text{F}$ .), the percentage of infertile chicken eggs increases (Heywang, 1944; Wilson, W. O., 1949); severe cold waves similarly tend to produce a decrease in the fertility of turkey (*Meleagris gallopavo*) eggs (Milby and Thompson, 1945). Restriction of mating activity is probably the primary factor contributing to poor fertility in hot or cold weather.

**The Influence of Management.** Various practices in poultry management may modify fertility, mainly by their effect on mating activity. The number of males to a flock, the freedom of movement permitted the birds, and similar factors can have a bearing on fertility.

**The ratio of males to females.** Studies of three breeds of chicken indicate that there is an inverse linear relationship between the percentage of fertile eggs laid and the number of females mated to one male (Byerly and Godfrey, 1937). The accompanying table shows the effect of the ratio of male to female chickens on fertility from the summarized data of Byerly and Godfrey (1937). A ratio of eighteen or twenty females to one Leghorn male and twelve to sixteen females to one male of other breeds has been recommended (Jeffrey, 1943; Parker and Bernier, 1950).

Number of Females (per male)	Fertility (per cent)
1- 50	93
51-100	73
101-150	38
151-200	43

The best fertility in ducks (*Anas platyrhynchos*) has been obtained when the ratio of ducks to drakes is 50:5. Fronda, Zialcita, and Dalisay (1940) stated that results become progressively poorer with ratios of 50:4 and 50:3, and are poorest when one drake is given twenty-five mates.

**Confinement of birds.** Kumanov (1948) reported a slightly higher percentage of fertile chicken eggs laid by confined flocks than by flocks allowed free range. The fertility of chicken eggs seems to be unaffected by confinement of the birds (Parker and Barton, 1946).



**Pen and stud mating.** Palafox (1948) noted that pen matings of White Leghorn pullets and cockerels yielded 80 per cent fertility, whereas stud matings of the same birds resulted in 60 per cent fertility. Nicolaides (1934), on the other hand, found no difference in the fertility of the two types of mating.

**Psychological Factors.** Mating with favorites to the exclusion of other birds is common among chickens of both sexes (*Philips, 1919; Upp, 1928; Warren and Kilpatrick, 1929; Skard, 1937; Hays and Sanborn, 1939; Lamo-reux, 1940a; Guhl, 1941, 1942; Guhl, Collias, and Allee, 1945; Guhl and Warren, 1946*). Upp (1928) observed that the daily matings per hen may range from zero to seven and that some hens may not mate for 3 to 8 consecutive days. Preferential mating, therefore, is an important factor contributing to infertility. It is especially prevalent among turkeys, *Meleagris gallopavo* (*Wilcke, 1939; Milby and Thompson, 1945*).

When several cocks run simultaneously with a flock of hens, the male social order may also interfere with mating activity. The dominating male curtails the activity of his inferiors to varying extents (*Guhl and Warren, 1946*).

### The Fertility of Intergeneric and Interspecific Matings and Hybrids

Many hybrids are reported to have occurred naturally among birds. Suchetet (1897) listed 234 individual museum specimens of birds which were reputedly hybrids, 124 being hybrids between species of the same genus and the remainder hybrids between species of different genera. Guyer (1909c), who made a similar survey of museums, found over sixty specimens of gallinaceous birds that represented about thirty different crosses, all but ten of which were intergeneric. Hybridization has also been performed experimentally, especially between different ducks (*Bonhote, 1905; Poll, 1910; Phillips, 1915, 1921*), pheasants (*Ghigi, 1907, 1908; Haig Thomas, 1909, 1912; Phillips, 1913, 1915, 1916, 1921; Smith and Haig Thomas, 1913; Haig Thomas and Huxley, 1927*), and pigeons or doves (*Whitman, 1919; Riddle, 1925; Painter and Cole, 1943*), as well as between canaries and various finches (*Plath, 1922; Bryan, 1945; and many others*). The development of techniques for the artificial insemination of birds has extended the possibilities for experimentation in this field.

In general, the closeness of the relationship between two species of birds determines whether or not union of the reproductive cells can occur, and, if it can, with what frequency. Many birds are so distantly related that fertility between them is impossible. Matings (natural, or achieved by artificial insemination) between less widely separated species yield variable percentages of fertilized eggs. The accompanying table, in which the male parent is given first, summarizes some of the observations on the fertility of such crosses.



Species or Genera Crossed	Eggs Fertilized (per cent)	Investigator
<i>Gallus gallus</i> × <i>Phasianus colchicus</i>	2	Smith (1912)
<i>Columba livia</i> × <i>Streptopelia decaocto</i>	86	Riddle (1925)
<i>Gennaeus nycthemerus</i> × <i>Gennaeus swinhoei</i>	100	Haig Thomas and Huxley (1927)
<i>Phasianus colchicus</i> × <i>Syrmaticus reevesii</i>	33	Haig Thomas and Huxley (1927)
<i>Phasianus colchicus formosanus</i> × <i>Phasianus versicolor</i>	13	Haig Thomas and Huxley (1927)
<i>Phasianus versicolor</i> × <i>Syrmaticus reevesii</i>	25	Haig Thomas and Huxley (1927)
<i>Chrysolophus pictus</i> × <i>Phasianus colchicus</i>	13	Haig Thomas and Huxley (1927)
<i>Columba livia</i> × <i>Streptopelia decaocto</i>	54-55	Cole, Painter, and Zeimet (1928b); Painter and Cole (1943)
<i>Gallus gallus</i> × <i>Numida meleagris</i>	11	Marchlewski (1937)
<i>Gallus gallus</i> × <i>Meleagris gallopavo</i>	20	Quinn, Burrows, and Byerly (1937)
<i>Gallus gallus</i> × <i>Numida meleagris</i>	8	Owen (1941a)
<i>Perdix perdix</i> × <i>Gallus gallus</i>	71	Bravo (1945)
<i>Gallus gallus</i> × <i>Gennaeus nycthemerus</i>	27-44	Marchlewski (1949)
<i>Columba livia</i> × <i>Columba guinea</i>	51	Taibel (1949)
<i>Gallus gallus</i> × <i>Chrysolophus pictus</i>	67	Marchlewski (1953)
<i>Phasianus colchicus</i> × <i>Meleagris gallopavo</i>	50	Asmundson and Lorenz (1955)

It should be noted that matings reciprocal to some of those listed in the table yield fertility of quite a different order. Artificial insemination of domestic hens with turkey (*Meleagris gallopavo*) semen results in fertilization of no more than 1 or 2 per cent of the eggs laid (Warren and Scott, 1935a; Quinn, Burrows, and Byerly, 1937), and matings of the male pheasant (*Syrmaticus reevesii*) to the female pheasant (*Phasianus versicolor*) are apparently entirely infertile (Haig Thomas and Huxley, 1927). Crossing the male pigeon (*Columba guinea*) and the female pigeon (*Columba livia*) results in average fertility of only 16 per cent (Taibel, 1949). On the other hand, better fertility (14 per cent as compared with 8 per cent) is obtained from the mating of the guinea cock (*Numida meleagris*) with the female chicken than from the reciprocal mating (Owen, 1941a).

Among birds as among mammals, many hybrids exhibit poor fertility or are completely sterile. Whitman (1919) noted that the fertility of hybrid wild pigeons is directly proportional to the closeness of the relationship between the parents' species. This observation is probably applicable to avian hybrids in general. The cause of impaired fertility in hybrids is irregularity in oögenesis or spermatogenesis, due in turn to incompatibility be-

tween the chromosomes of maternal and paternal origin (see Chapter 1). A partial list of observed sterile hybrids is given herewith (the male parent is named first).

Species Crossed	Sex of Sterile Hybrid	Investigator
<i>Cairina moschata</i> × <i>Anas platyrhynchos</i>	M, F	Poll (1910); Cavazza (1931); Sokolovskaia (1935a); Crew and Koller (1936)
<i>Anas platyrhynchos</i> × <i>Cairina moschata</i>	M	Poll (1910); Crew and Koller (1936)
<i>Columba livia</i> (Magpie pigeon × domestic dove)	M	Smith (1912)
<i>Syrnaticus reevesii</i> × <i>Phasianus colchicus</i>	F	Phillips (1913)
<i>Phasianus colchicus</i> × <i>Syrnaticus reevesii</i>	F	Phillips (1913)
<i>Phasianus colchicus formosanus</i> × <i>Syrnaticus reevesii</i>	M, F	Smith and Haig Thomas (1913)
<i>Phasianus versicolor</i> × <i>Syrnaticus reevesii</i>	M, F	Smith and Haig Thomas (1913)
<i>Phasianus colchicus principalis</i> × <i>Syrnaticus reevesii</i>	M, F	Phillips (1916)
<i>Syrnaticus reevesii</i> × <i>Phasianus colchicus principalis</i>	M, F	Phillips (1916)
<i>Phasianus colchicus</i> × <i>Gallus gallus</i>	M, F	Cutler (1918)
<i>Carpodacus mexicanus</i> × <i>Serinus canaria</i>	M	Plath (1922)
<i>Zenaidura macroura</i> × <i>Streptopelia decaocto</i>	F	Riddle and Johnson (1939)
<i>Gallus gallus</i> × <i>Numida meleagris</i>	M	Owen (1941a)
<i>Gallus gallus</i> × <i>Gennaeus nycthemerus</i>	F	Marchlewski (1949)
<i>Columba livia</i> × <i>Columba guinea</i>	F	Taibel (1949)
<i>Columba guinea</i> × <i>Columba livia</i>	F	Taibel (1949)
<i>Phasianus colchicus</i> × <i>Meleagris gallopavo</i>	M, F	Asmundson and Lorenz (1955)
<i>Meleagris gallopavo</i> × <i>Phasianus colchicus</i>	M, F	Asmundson and Lorenz (1955)

In addition, Whitman (1919) observed complete and partial sterility in certain hybrid wild pigeons. Partial sterility has been noted (Ghigi, 1907, 1908; Phillips, 1921) in hybrids of the silver pheasant (*Gennaeus nycthemerus*) and Swinhoe's pheasant (*Gennaeus swinhoei*).

The fertility of eggs obtained from wide crosses is distinct from their ability to hatch. Embryonic mortality in such eggs is usually high and may attain 100 per cent.



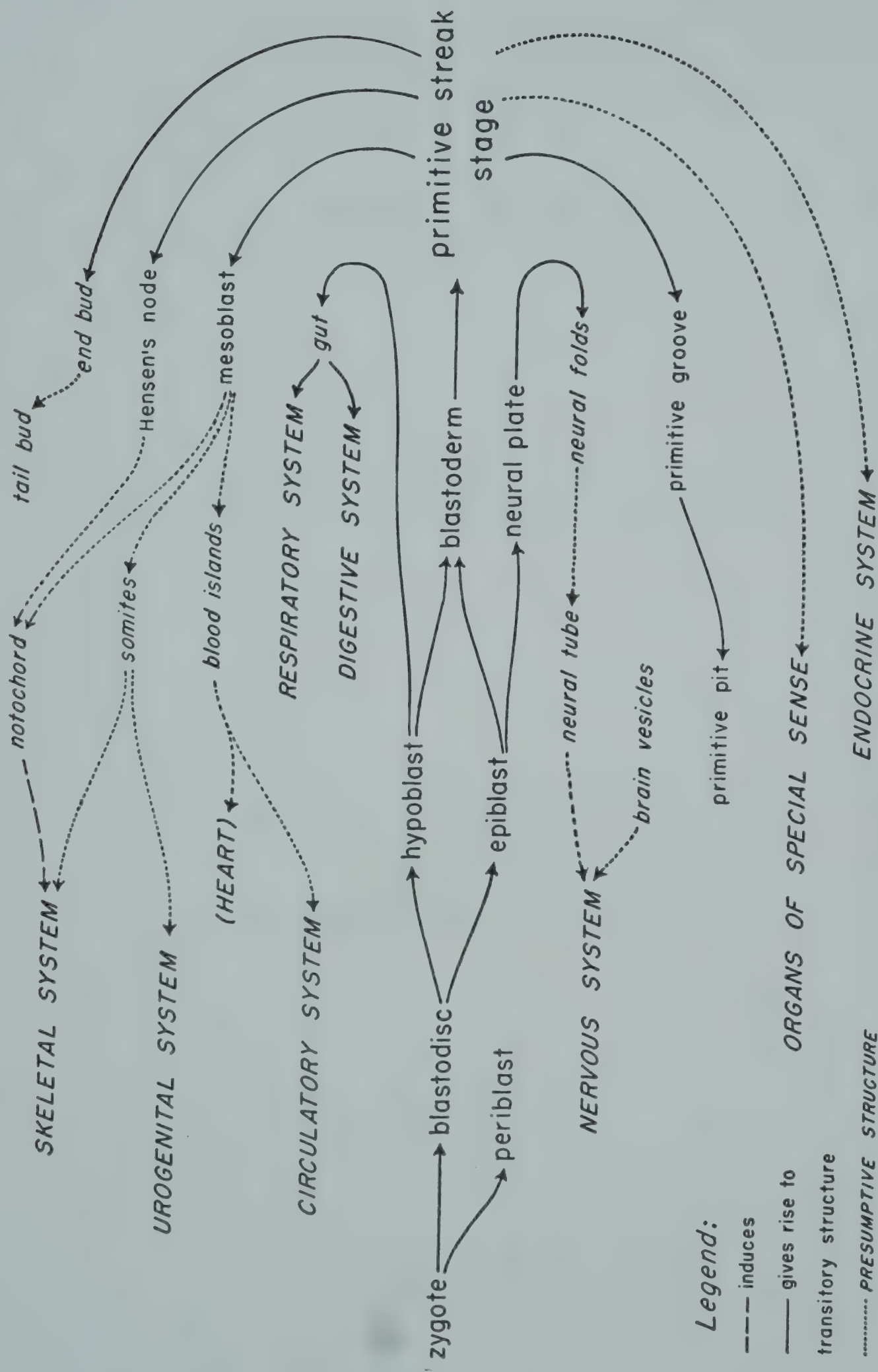


## CHAPTER THREE

# *Early Morphogenesis*

*A single cell, by its division,  
Becomes three layers most unique—  
Organization with precision  
Which forms an embryonic streak.*





EARLY MORPHOGENESIS IN THE AVIAN EMBRYO

## EARLY MORPHOGENESIS

The first visible step in the development of the avian embryo is the transformation of the zygote from the unicellular to the multicellular state. Almost from the beginning, however, embryogenesis is advanced not only by cell division but also by the movement of cell masses. These two processes continue for some time without establishing any recognizable organs or definite organ primordia; only very simple structures are formed, and only very general cellular differentiations occur.

The period prior to organogenesis is nevertheless morphogenetic. It is during this time that the future developmental direction of many cells and groups of cells is determined. Also, as cellular migration continues, it becomes clear that there is a definite relationship between the embryonic body and the primitive structures formed during the early hours of incubation. The initial stages of development, therefore, are critical.

### CLEAVAGE OR SEGMENTATION

After the formation of the segmentation nucleus from the union of the male and female pronuclei (see Chapter 2), the ovum is carried down the oviduct by peristalsis. On its way, it becomes invested with several layers of albumen, over which are applied the shell membranes and finally the calcareous shell (*Romanoff and Romanoff, 1949, pp. 175–252*). By the time the egg has reached structural completion, development of the embryo has proceeded to a multicellular stage and the second of the three primary “germ layers” has usually begun to appear.

The sequence of cell divisions that initiates embryonic development is known as segmentation, or cleavage. Two general types of segmentation occur in animal eggs: holoblastic, or complete, characterized by division of the entire ovum; and meroblastic, or partial, in which cell divisions are restricted to a certain part of the egg. The type of cleavage is related to the disposition of yolk. Homolecithal eggs, in which yolk is distributed more or less uniformly, undergo holoblastic cleavage. Meroblastic cleavage is found in telolecithal eggs, where the relatively large amount of yolk is concentrated at one pole and the living cells are confined to the other; only



the protoplasmic portion of the egg takes part in cleavage. Birds' eggs, being telolecithal, undergo meroblastic segmentation.

### The First Division

Segmentation begins not long after fertilization. The usual interval is about 5 hours in eggs of the chicken (Olsen, 1942), the turkey, *Meleagris gallopavo* (Olsen and Fraps, 1944), and the pigeon, *Columba livia* (Blount, 1909), although it has been stated that a shorter time (about 3 hours) sometimes elapses between fertilization and the beginning of cleavage in both the pigeon's egg (Harper, 1904) and the hen's egg, *Gallus gallus* (Patterson, 1910). Since the length of the oviduct and the speed of the egg's passage through it vary somewhat from one bird to another of the

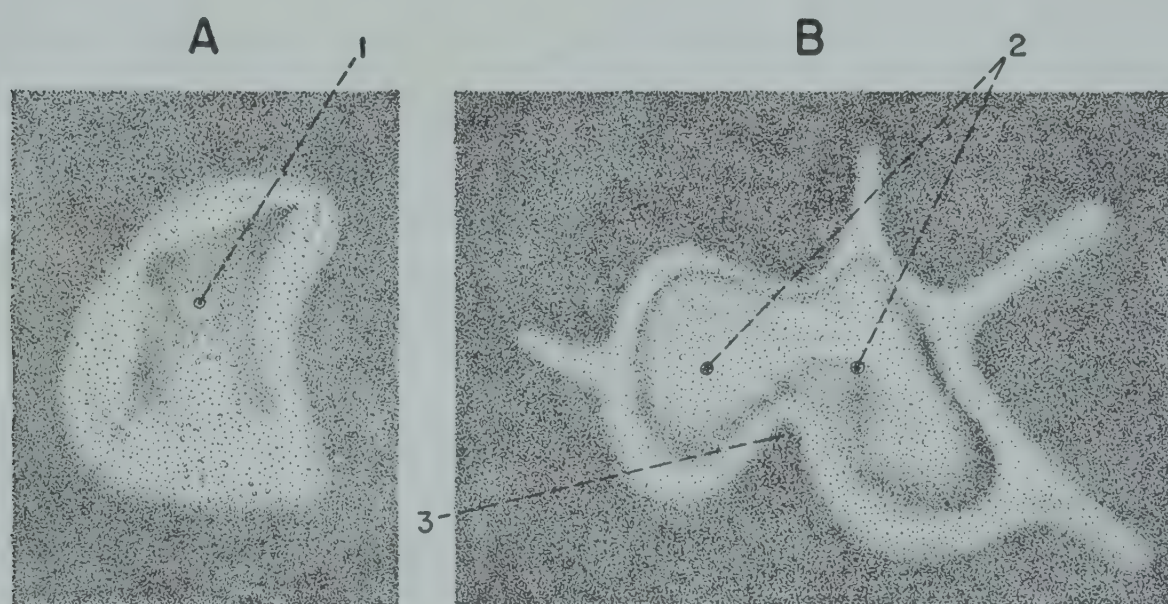


Fig. 42. The first segmentation stages in the pigeon's (*Columba livia*) egg, as seen in horizontal sections. (Redrawn with modifications after Harper, 1904.)

A, the first cleavage spindle and its surroundings (from an egg found in the shell membrane-secreting region of the oviduct); B, the first pair of cleavage nuclei and their surroundings. Both  $\times 65$ .

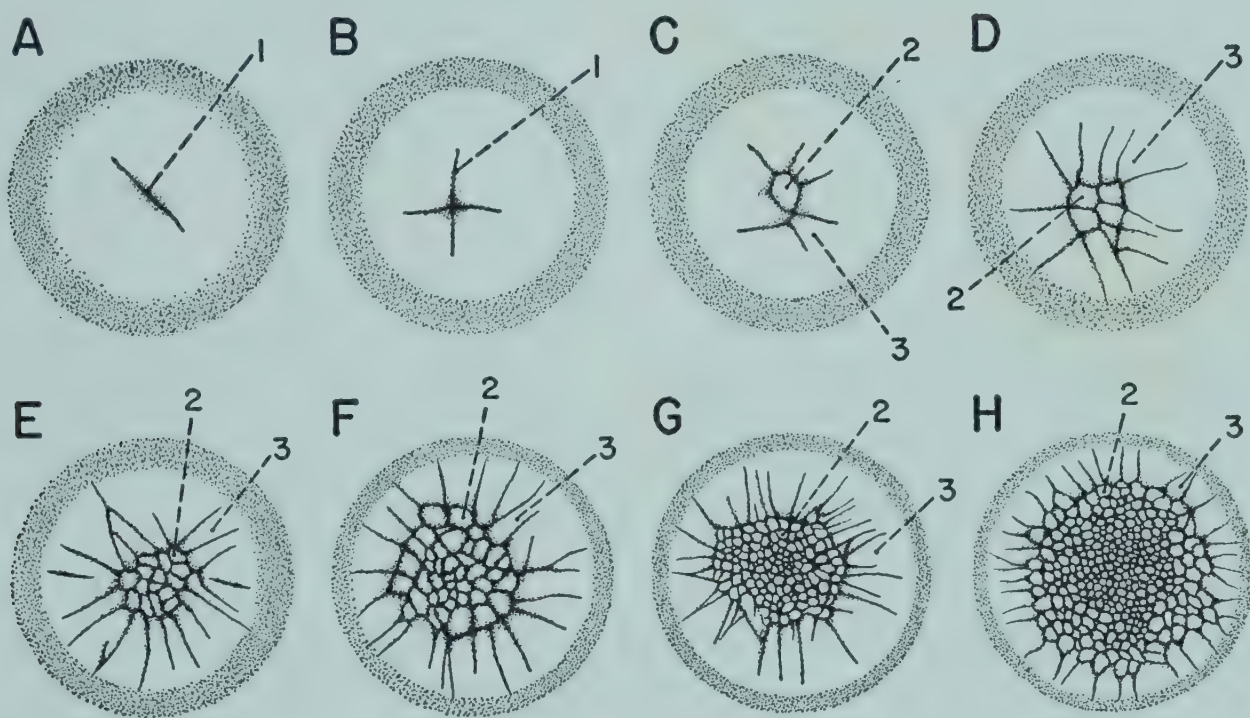
1, first cleavage spindle; 2, first pair of cleavage nuclei; 3, indentation indicating region of first cleavage furrow.

same species, the stage of cleavage is not always exactly the same at the same oviducal level in different individuals. Segmentation may start when the egg is in any part of the oviduct from the more posterior sections of the albumen-secreting portion (Beneden, 1870) to the uterus, or shell gland. In general, the first segmentation division takes place while the egg is in the isthmus (which secretes the shell membranes), as indicated many years ago by Coste (1850a) and Kölliker (1879, p. 69).

The earliest stages of cleavage have been described in detail by Harper (1904), who used the pigeon's (*Columba livia*) egg as experimental material. He laid considerable stress upon the differentiation of the "activated" portion of the cytoplasm (that is, the portion immediately surrounding the nuclear material) into an inner granular and a peripheral hyaloplasmic



area (Fig. 42-A). Although other workers have not emphasized this phenomenon in birds' eggs, their photographs indicate that the cytoplasm of the first cleavage cells, or blastomeres, is not homogeneous (*Durme, 1914; Olsen, 1942; Olsen and Fraps, 1944*). As noted in Chapter 2, Harper (1904) observed modification of the cytoplasm at the time the segmentation nucleus forms. He also called attention to the presence of amoeboid outpushings at the hyaloplasmic margins of the affected cytoplasmic area after the formation of the first cleavage spindle. At this time, there is a gradual migration of the "wedge" granules of the periblast away from the side of the blastodisc that has been predetermined as the anterior portion (*Bartelmez, 1912*). These granules move to the posterior margin of the blastodisc, where they form a crescent.



**Fig. 43.** Surface views of the chick (*Gallus gallus*) blastoderm during segmentation. (Redrawn with modification after Patterson, 1910.)

A to H show the increases in the number of visible cells from the time of the first division to that of an advanced stage. All  $\times 8$ .

1, cleavage furrow; 2, central cell; 3, marginal cell.

Figure 42-B is a horizontal or tangential section taken through the active portion of the pigeon's (*Columba livia*) blastodisc soon after the division of the segmentation nucleus. The first pair of cleavage nuclei are present, and a transverse constriction indicates the location of the first cleavage furrow. Harper (1904) considered one of these cleavage cells to be more hyaline than the other, although the difference is not pronounced in the figure. He found that the next division is also selective, a more hyaline and a more granular blastomere being formed from each of the original cells. The literature does not contain other statements to this effect, however.

Segmentation occurs at the surface of the germinal area. When this area is seen from above, the first cleavage furrow appears as a straight line crossing the central portion, which is the segmental disc, or blastodisc. As



shown in Fig. 43-A, depicting the chicken blastodisc, the furrow does not extend to the border (the periblast), nor does it, in fact, traverse the entire width of the inner zone. There appears to be no constant relationship between the direction of the first cleavage plane and the future longitudinal axis of the embryo (Blount, 1909; Patterson, 1910; Olsen, 1942).

The first cleavage plane, furthermore, does not extend throughout the entire depth of the blastodisc (Fig. 44-A). The first two blastomeres are incompletely delimited cells, for they are continuous with the yolk below and with the periblast peripherally.

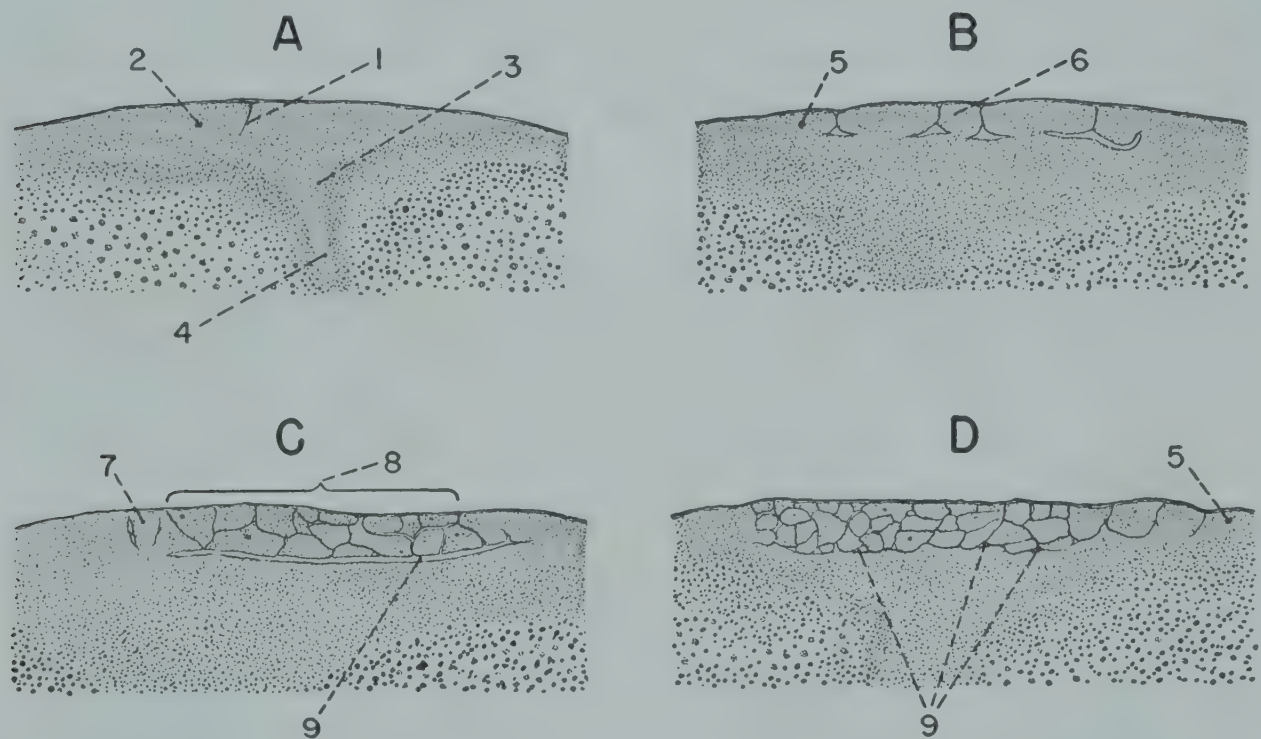


Fig. 44. Sections through the blastodisc of a chicken (*Gallus gallus*) egg during segmentation. (Redrawn with modifications after Patterson, 1910.)

A, vertical section transecting the first cleavage furrow; B, median longitudinal section through the eight-celled blastodisc; C, the same section through a blastodisc with sixty-four cells visible superficially; D, the same section through a blastodisc with 154 cells visible superficially. All  $\times 22$ .

1, cleavage furrow; 2, cleavage nucleus; 3, central periblast; 4, neck of latebra; 5, marginal cell nucleus; 6, blastomere; 7, marginal blastomere; 8, central blastomeres; 9, segmentation cavity.

Cross sections of the blastodisc at the two-celled stage show, in addition, that the first cleavage furrow may, or may not, indent the surface of the blastodisc and that it may be sharply defined or very diffuse. Although Patterson (1910) found the first furrow to be much less distinct in the chicken's egg than in the pigeon's (*Columba livia*), subsequent work indicates that its appearance is probably a characteristic of the individual egg rather than of the species (Olsen, 1942; Olsen and Fraps, 1944).

### The Four-Celled Stage

In the chicken egg, the first two blastomeres divide within a very short time—probably not more than 15 minutes—after their formation (Patter-



son, 1910; Olsen, 1942). In the pigeon (*Columba livia*) egg, an interval of 1 to 2 hours apparently elapses between the first and second cleavage divisions (Harper, 1904; Blount, 1909). Usually, but not always, the two cells divide simultaneously. Coste (1850a) found that the four-celled stage in the hen's egg coincides with the initial appearance of the inner shell membrane as a thin, diaphanous pellicle surrounding the albumen.

Like the first cleavage furrow, the second is in a vertical plane and does not reach the limits of the blastodisc. It meets the first furrow at a right angle (Fig. 43-B), so that—as Coste (1850a) remarked—the two furrows are seen as a Maltese cross in surface views of the blastodisc. However, the cross is very frequently irregular, for the furrow subdividing one of the first two blastomeres may not be continuous with the furrow that subdivides the other.

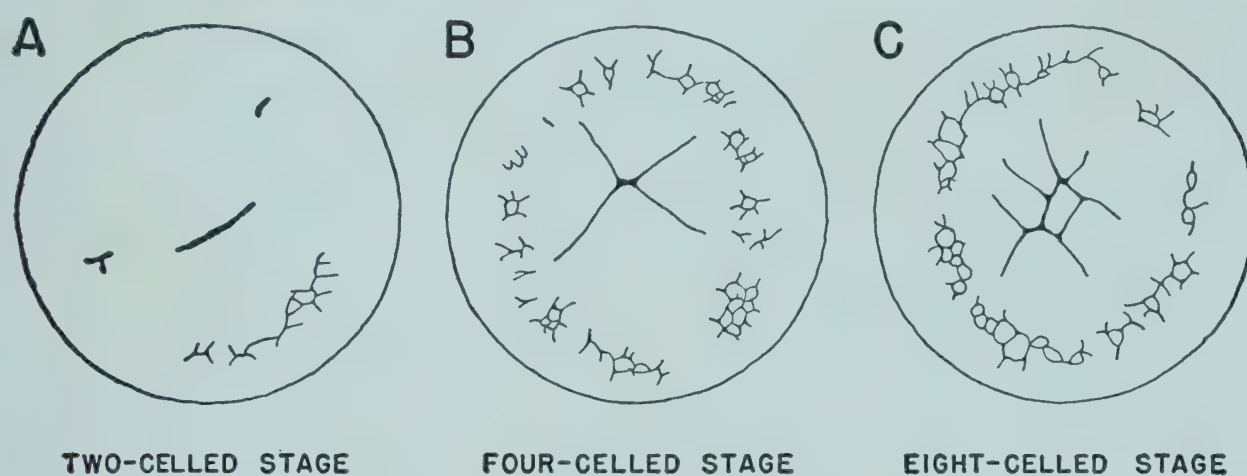


Fig. 45. Diagrammatic surface views of pigeon (*Columba livia*) blastodiscs, showing the increasing number of accessory cleavage cells during early stages of segmentation. (Redrawn with modifications after Harper, 1904.) All  $\times 7$ .

When the four-celled stage is reached, it often becomes apparent that the cleavage center is located eccentrically on the blastodisc. Earlier workers (Kölliker, 1879, p. 79; Duval, 1884a; Kionka, 1894) stated that segmentation always begins at one side of the blastodisc; Duval (1884a) and Kionka (1894) claimed, furthermore, that the initial divisions invariably occur in the posterior portion of the segmental disc. Later investigations, however, have shown that cleavage is frequently symmetrical and that, if it is eccentric, it may be displaced in any direction without relationship to the future orientation of the embryo (Patterson, 1910; Olsen, 1942).

At the four-celled stage, segmentation of the accessory sperm cell nuclei (if these are present) becomes clearly apparent (Fig. 45). As noted in Chapter 2, some of these nuclei begin to migrate toward the periphery of the germinal disc at the time of the conjugation of the male and female pronuclei. Other sperm nuclei drop down through the substance of the blastodisc into the subjacent yolk, which has been called the "central periblast." The nuclei in the deeper location degenerate, but those around the margins of the blastodisc divide and initiate a rudimentary type of cleavage



that is entirely transitory in nature. The accessory cleavage planes are first seen as short radial furrows between the blastodisc and the periblast (*Blount, 1909; Patterson, 1910*). In the turkey (*Meleagris gallopavo*) egg, the four-celled stage is the only period in which accessory cleavage has been found (*Olsen and Fraps, 1944*).

### The Eight-Celled Stage

In the hen's egg, the blastomeres of the four-celled stage divide about an hour after their formation (*Patterson, 1910; Olsen, 1942*), or approximately at the time the shell membrane becomes opaque (*Coste, 1850a*). In the pigeon's (*Columba livia*) egg, about 1.5 hours pass before division occurs (*Blount, 1909*). The eight daughter cells usually form an irregular pattern (Fig. 43-C), but they are found in two parallel rows if the cleavage furrows added at this time are parallel with the first furrow. The new furrows, like the first and second, do not reach the edge of the blastodisc. Frequently, one of the eight blastomeres may be completely surrounded (in surface view) by furrows (cf. Fig. 43-C), but all the cells of this stage are still continuous with the underlying yolk. As Fig. 44-B shows, however, the first signs of horizontal cleavage planes are appearing beneath the blastomeres, which are thus partially cut off from the yolk below.

The eight-celled stage is the last in which accessory cleavage has been seen in the chicken egg (*Patterson, 1910; Olsen, 1942*). The supernumerary sperm nuclei seem incapable of further division, and they degenerate after this period. Those in the "central periblast" lose their power of migration shortly after taking up their deeper position and degenerate even sooner than those that remain in a superficial location (*Patterson, 1910*).

In the pigeon's (*Columba livia*) egg, on the other hand, the accessory sperm nuclei may have divided and increased considerably in number by this stage (cf. Fig. 45-C). Accessory cleavage planes, therefore, may form a peripheral limit to most of the cells of the segmental disc (*Blount, 1909*).

### The Sixteen-Celled Stage

In the hen's egg, about 45 minutes are required to make the transition from the eight-celled to the 16-celled stage (*Patterson, 1910; Olsen, 1942*); in the pigeon's (*Columba livia*) egg, about twice as much time is needed (*Blount, 1909*). The chicken egg, like the turkey (*Meleagris gallopavo*) egg (*Olsen and Fraps, 1944*), has usually moved into the uterus by the time the blastodisc is composed of 16 cells. *Patterson (1910)*, however, found that the number of cells may vary from 11 to 34 at the time the hen's egg enters the uterus, and *Kölliker (1879, p. 73)* indicated that as many as 44 may be present. According to *Coste (1850a)* and *Beneden (1870)*, the deposition of the first calcareous material of the shell coincides with the establishment of the 16-celled blastodisc.



Two noteworthy changes may be found in the blastodisc with 16 cells. First, divisions have formed new cells from the medial portion of each blastomere (*Beneden, 1870*). When the blastodisc is seen from above, the cells in the center of the cleavage area appear completely surrounded by walls, and the blastodisc itself is now differentiated into a central and a marginal zone. The cells of the marginal area are still incompletely delimited blastomeres divided from each other by furrows radiating outward (Fig. 43-D). Some of these radial furrows may now reach the edge of the blastodisc. Around the margin of the cleavage area are sometimes short radial furrows which foreshadow the peripheral extension of the longer radial furrows and which perhaps also indicate where the next divisions of the marginal cells will occur (cf. Fig. 43-D). These furrows are not to be confused with accessory cleavage furrows, for they are confined to the segmental disc and do not cut across its borders into the periblast.

The second change visible in the 16-celled blastodisc appears in cross sections. The horizontal cleavage planes now extend beneath the cells of the central area and, through confluence, have formed a small, slitlike fissure. The central cells are completely separated from the yolk by this narrow space, which has been termed the segmentation cavity, or blastocoele (*Duval, 1884a; Kionka, 1894; Blount, 1909; Patterson, 1909b, 1910*), probably erroneously (*Pasteels, 1945*).

### Later Stages

After the 16-celled stage, the cells of the marginal area multiply, and additional radial furrows are formed (Fig. 43-E). Also, new cells are cut off from the marginal cells and are added to the central area, which thus grows at the expense of the marginal area (*Patterson, 1910*).

In the pigeon's (*Columba livia*) egg, accessory cleavage persists through the 32-celled stage (*Blount, 1909*). The accessory sperm nuclei undergo repeated division and establish a more or less continuous zone of small cells, two or three layers deep, between the blastodisc and the periblast. Except where this zone is lacking, the marginal cells of the primary area are separated from the periblast by incomplete cleavage planes running diagonally downward, as well as by the accessory cells. A few subgerminal sperm nuclei may be found near the margins of the nucleus of Pander. These nuclei and the accessory cleavage cells begin to degenerate very shortly after this stage, and the marginal cells of the blastodisc again become open to the periblast below and peripherally.

After the 32-celled stage, the increase in the number of cells in the central area is greatly accelerated by the division of the central cells themselves, while additions from the margin continue. Figure 43-F depicts a blastodisc containing 64 cells in surface view. It will be noted that the innermost cells of the medial zone are considerably smaller than those lying just inside



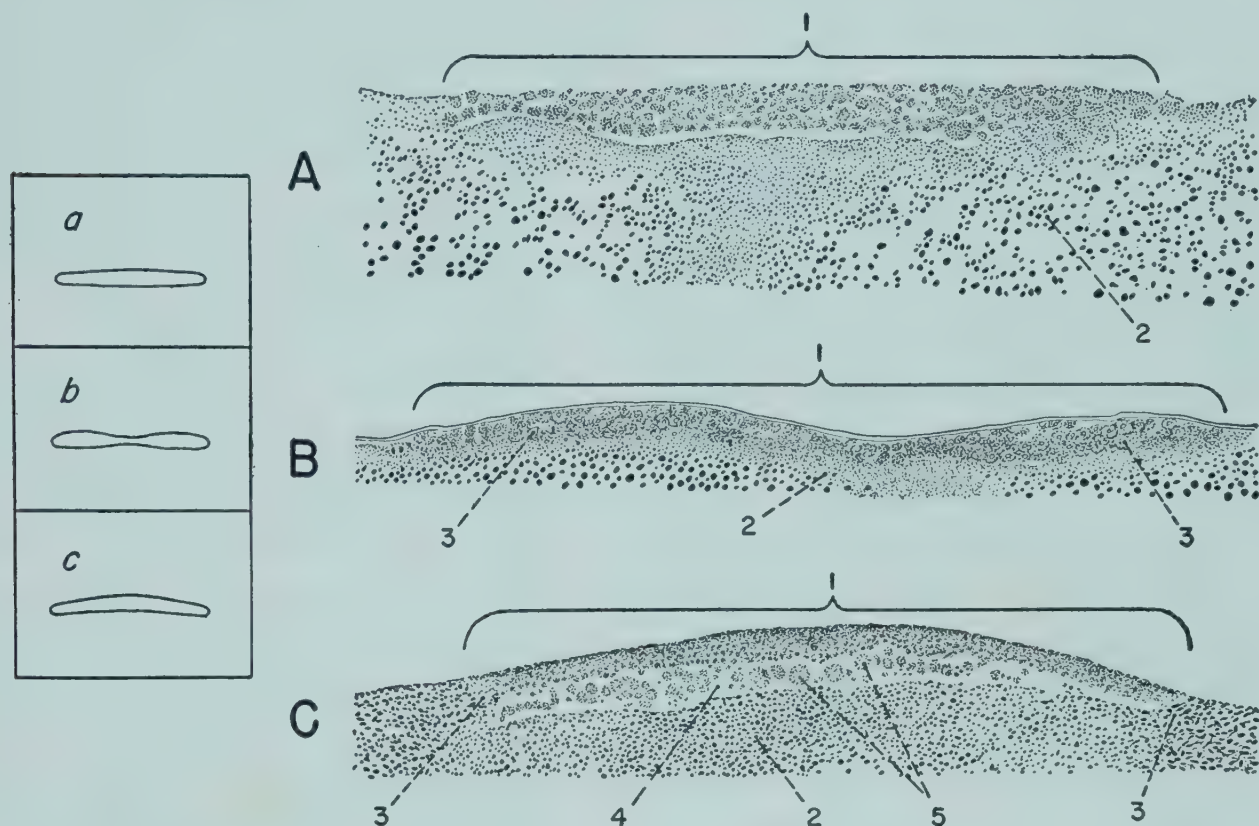
the marginal zone, a fact which indicates recent division of the most centrally located cells. Furthermore, the blastodisc represented in this figure actually contains more than the 64 cells visible superficially, for the central cells have undergone both horizontal and vertical division. The central area of this blastodisc is two cells deep (Fig. 44-C) and is separated from the yolk by a clear-cut fissure.

Both Kölliker (1879, *p.* 75) and Duval (1884*a*) attributed the increase in the number of layers in the central area to the addition of cells upward from the yolk. Kölliker believed that nuclei pass down into the yolk from the original layer of cells and divide, new cells being organized around the more superficial daughter nuclei. Through the repetition of this process, cleavage would proceed downward. Duval's theory was similar, except for the fact that he placed the segmentation cavity directly beneath the uppermost layer of cells. Duval's studies of cleavage were made on eggs that were supposedly unfertilized and undergoing parthenogenetic development; his results are at best ambiguous. Furthermore, Patterson (1910) stated that, at this stage, there are no nuclei in the yolk beneath the blastodisc. Blount (1909) and Patterson (1910) agreed that the central area of the blastodisc becomes stratified through the formation of horizontal planes of division above the first horizontal plane; that is, by division of the cells making up the original layer. This view, which has never been disputed subsequently, is substantiated by the observation that the cells making up the two-layered disc are much smaller than those of the original single layer. Also, the total depth of the central part of the blastodisc is practically the same both before and after the development of the second layer of cells. Figure 44-D shows that the blastodisc with three layers of central cells is but little thicker than the single-layered germ and that the cells are still smaller than those in the two-layered disc.

As new layers of cells are added to the central zone, it eventually increases in thickness. At the same time, it continues to grow wider by adding to its periphery cells derived from material of the marginal zone (Fig. 43-G and H). The latter becomes progressively narrower and finally disappears entirely. In surface view, the blastodisc now appears to be composed of a continuous sheet of small cells, hence it becomes known as the blastoderm. Kionka (1894) noted that the blastoderm of this period appears biconvex in cross section, and that the proliferation of cells (to a depth of six layers at the thickest point) may have virtually obliterated the cavity beneath the central area (Fig. 46-A). Pasteels (1945) termed the duck's (*Anas platyrhynchos*) blastoderm at this stage an "advanced morula"; that is, he considered it equivalent to the solid mass of cells which, in other forms, precedes the formation of the hollow blastula. It is incompatible with this view, of course, to regard as a blastocoele the cavity which forms beneath the avian blastodisc at the 16-celled stage.



Before the marginal area of the blastodisc is completely subdivided into cells, the radial cleavage furrows passing through it begin to cut into the periblast (cf. Fig. 43-H). This extension of the field of activity can be observed within a few hours after the egg has entered the hen's uterus, at a time when there are about 350 cells visible in a surface view of the blastodisc (*Patterson, 1910*). As soon as the marginal area disappears, the periblast begins to be organized into cells. Cellularization of the periblast begins in the chicken egg about 14.5 or 15.5 hours after fertilization (*Sturkie and Williams, 1945*).



**Fig. 46.** Three successive stages in the development of the avian blastoderm. (Redrawn with modification A, after Pasteels, 1945; B, after Patterson, 1909b; C, after Peter, 1938b.)

A, a section through the blastoderm of a duck's (*Anas platyrhynchos*) egg, showing the biconvex shape characteristic of late segmentation; B, a median longitudinal section through a pigeon (*Columba livia*) blastoderm at a somewhat later stage (21 hours after fertilization), when the central portion of the blastoderm is growing thinner; C, section through a chicken (*Gallus gallus*) blastoderm of a still later stage, when the blastoderm is concavo-convex. All  $\times 65$ .

*Insert:* diagrams showing changes in the shape of the developing blastoderm at stages corresponding to those shown in A, B, and C.

1, blastoderm; 2, yolk; 3, germ wall; 4, subgerminal cavity; 5, yolk spheres.

Blount (1909) stated that cells arise in the peripheral and central periblast around nuclei that migrate outward and downward from the periphery of the blastoderm, under which the segmentation cavity does not extend. In this manner, there is created a nucleated syncytium that forms a zone of junction between the periblastic yolk and the margin of the blastoderm. Cells are cut off from the inner portion of the syncytium and are added to the periphery of the blastoderm (*Patterson, 1909b*). The region where new



cells are displaced centripetally out of the zone of junction and into the blastoderm is known as the germ wall. It should be remarked at this point that the syncytial nature of the zone of junction has been denied by Peter (1938c) and a number of other investigators. If the syncytium does not exist, then the entire marginal region may be considered as germ wall.

The zone of junction expands over the yolk like a widening ring, adding new cells from its inner border to the outer margin of the blastoderm proper. As a result of this addition of cells, the blastoderm begins to grow. Its diameter has increased very little—perhaps by about 0.2 mm. (Kionka, 1894)—since the start of cleavage, when it measured less than 3.0 mm. (in the chicken egg). Now, however, it enlarges rapidly. The fact that growth takes place at the margin of the blastoderm, rather than farther centrally, has been shown by experiments on somewhat older blastoderms. Cauterization at the zone of junction prevents further growth at the site of injury, but cauterization more medially has no effect on the expansion of the blastoderm (Schlesinger, 1952).

Peripherally, the blastoderm soon thins out toward its surface and may taper to a thickness of only one cell, since the addition of new cells from the zone of junction is most rapid superficially. This thin margin has been called the “margin of overgrowth” (Blount, 1909), because it grows directly over the yolk. The blastoderm also has a tendency to become thinner centrally than it is in the vicinity of the germ wall (Fig. 46-B). Kölliker (1879, p. 80) attributed this change to multiplication of the superficial cells and shifting of the deeper elements toward the margin.

The blastoderm of the pigeon's (*Columba livia*) egg reaches this stage of development at some time between the twentieth and the thirtieth hour after fertilization (Patterson, 1909b; Peter, 1938c). Peter (1938c) remarked that the blastoderm is now composed of round cells, those at the surface being the smallest. The germ wall may be very slightly thicker in one region than elsewhere; the subsequent course of development shows the thickened portion to be located posteriorly.

Cross sections of the blastoderm in a somewhat more advanced stage reveal that it has lost its biconvex shape and has become concavo-convex; that is, its external surface is still convex, but its internal surface is now concave (Fig. 46-C). The central portion has become still thinner, especially anteriorly, and now arches over a well-defined cavity (Duval, 1884a; Kionka, 1894; Peter, 1938b, 1938c; Patterson, 1909b), in which large, round masses of yolk may be seen (Goette, 1874). The thickness of the posterior germ wall has increased (Peter, 1938b, 1938c).

Because of these structural modifications, the blastoderm is differentiated visibly into two concentric areas (Fig. 47-B). The thin medial region is translucent; in surface view, by reflected light, it appears as the dark area pellucida. The thickened margin (or germ wall), lying on the yolk,



forms a light peripheral ring, the area opaca. No anteroposterior difference in the superficial appearance of the blastoderm is apparent at this time. The contours of both germinal areas may show considerable irregularity, especially in the pigeon's (*Columba livia*) egg (Peter, 1938c).

The cavity underlying the blastoderm at this stage has been called the subgerminal cavity, to distinguish it from the segmentation cavity formed at the 16-celled stage. Since both cavities occupy the same position between the blastoderm and the yolk, the difference is merely nominal, unless

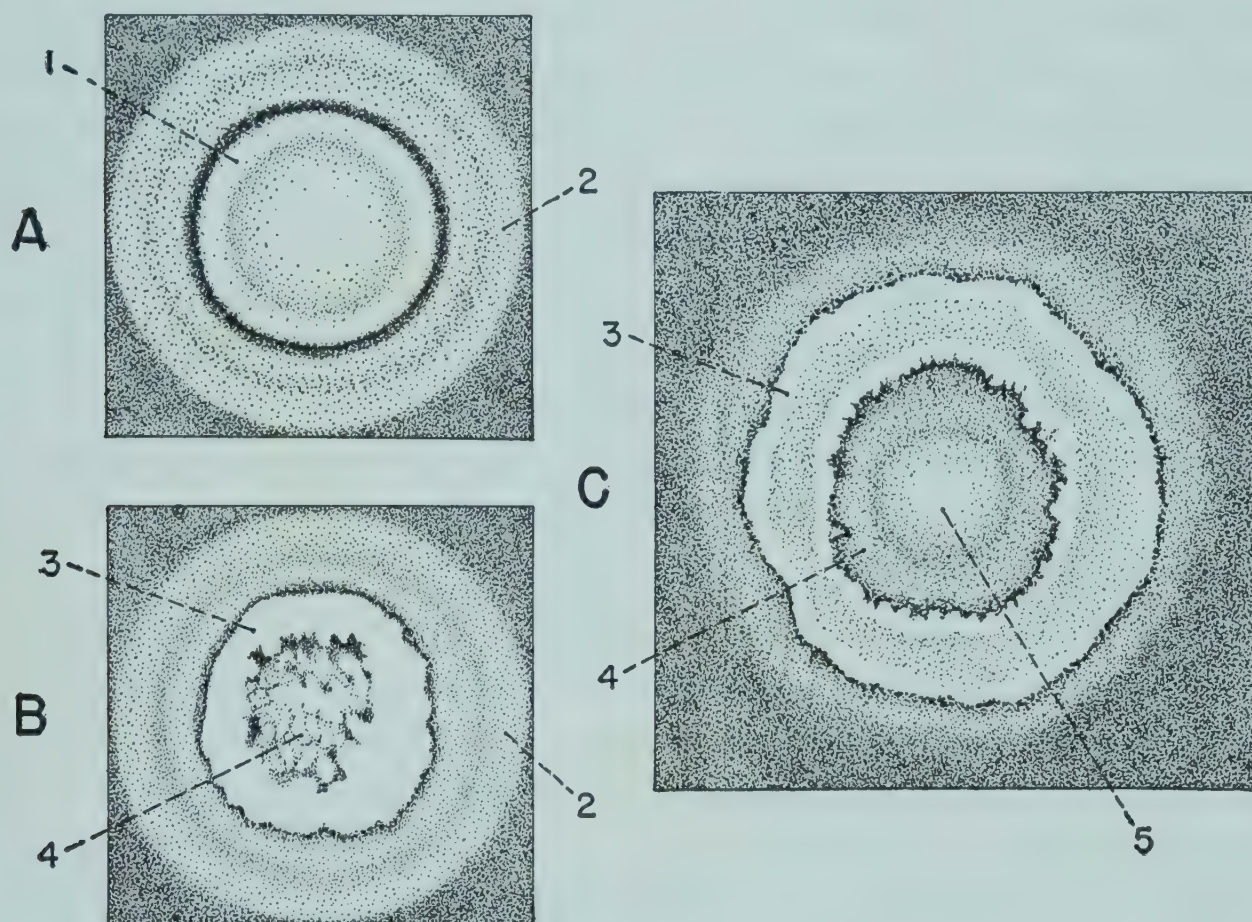


Fig. 47. The differentiation of the blastoderm into area pellucida and area opaca, as seen in surface views of blastoderms of pigeons' (*Columba livia*) eggs. (Redrawn with modifications after Peter, 1938c.)

A, appearance of the blastoderm about 30 hours (estimated) after fertilization; B, the blastoderm soon after differentiation into areas opaca and pellucida; C, the blastoderm about 42 hours after fertilization. All  $\times 7$ .

1, blastoderm; 2, periblast; 3, area opaca; 4, area pellucida; 5, nucleus of Pander showing through the area pellucida.

the subgerminal cavity is a new formation. Patterson (1909b) apparently considered the later cavity to be an enlargement of the earlier one. Kionka (1894) admitted that the subgerminal cavity appears to result from the reopening of the segmentation cavity, but he was inclined toward the opinion that it is entirely new in origin. Pasteels (1940, 1945) suggested that this new origin is the liquefaction of the yolk by enzymes present in either the yolk or the blastomeres. However, Pasteels denied that the subgerminal cavity is a true embryonic cavity; according to his view, it is merely a liquid-filled space.



It is of interest to note that new-laid hens' eggs containing concavo-convex blastoderms have been obtained after the application of semen directly on the surface of the ovary (*Olsen, 1952*) (see Chapter 2).

### Parthenogenetic Segmentation

Parthenogenesis, or the development of an individual from an unfertilized egg, is a type of reproduction occurring naturally in various invertebrates. Artificial stimuli (physical or chemical) may initiate embryonic development in the unfertilized eggs of certain animals that normally reproduce bisexually. This fact seems to indicate that some impetus is given to the egg by the sperm cell, an impetus that is quite apart from the mere contribution of protoplasm. There is, also, a further implication: that the unfertilized egg itself possesses the potential capacity for development, at least up to a certain stage. This inference is borne out by observations of rudimentary segmentation in infertile eggs of a number of animals. For many years, it has been debated whether or not the unfertilized eggs of birds undergo such "abortive" segmentation.

The investigators who have studied the blastodisc of the infertile, new-laid avian egg have almost universally agreed that structures resembling blastomeres may be present. Some authors believed that these structures are cellular in nature (*Oellacher, 1872; Motta Maia, 1877; Lécaillon, 1910a; Bartelmez and Riddle, 1924; Kosin, 1945; Marsden and Olsen, 1954*), whereas others regarded them as representing a vitelline fragmentation, the result of a purely physicochemical degenerative process (*Lau, 1894; Barfurth, 1896; Bonnet, 1900, 1907; Hays and Nicolaides, 1934*). Those who have denied the possibility of parthenogenetic segmentation either failed to find nuclei or suggested that the eggs in which nuclei were observed were actually fertile. The criticism that the origin of the experimental material was not well controlled appears to be justified for the work of Duval (1884a) and possibly for that of Oellacher (1872), but it does not apply to investigations undertaken since 1900, such as those of Lécaillon (1908a, 1908b, 1909a, 1909b, 1909c, 1909d, 1909e, 1909f, 1910a, 1910b, 1910c, 1910d, 1910e, 1910f, 1910g). In general, the weight of the evidence appears to favor the possibility that parthenogenetic segmentation, ending in degeneration, can occur.

According to Oellacher (1872), who studied oviducal chicken eggs, parthenogenetic segmentation begins very much as does normal cleavage. Apparently, however, cleavage in the infertile blastodisc either starts later or proceeds much more slowly than in the fertile egg, for segmentation in the very early stages in hard-shelled uterine eggs was observed by Oellacher and also by Chappellicr (1912b), who worked with hybrid ducks (*Anas platyrhynchos* male mated with *Cairina moschata* female).

Cross sections show that the segmental elements in the new-laid infertile



egg are of irregular size and form a compact mass, sometimes several layers deep centrally (Oellacher, 1872; Lécaillon, 1910a; Bartelmez and Riddle, 1924). The blastodisc is thus in a stage corresponding to that of the biconvex blastoderm, reached in the fertile egg some time before the latter is laid. There is no true subgerminal cavity, but the researches of Bartelmez and Riddle (1924) on the pigeon (*Columba livia*) egg appear to indicate that there may be some fluid beneath the segmented mass, because of the fact that the yolk absorbs water from the albumen.

The presence of nuclei in infertile blastodiscs is the most convincing evidence that cell division actually occurs. Kosin (1945) found nuclei in about 25 per cent of new-laid, infertile eggs, but he observed mitotic figures in only 16 per cent. Nuclei have been seen in the segmented portion and in the periblast also (Lécaillon, 1910a; Chappellier, 1912b). Many of the nuclei of the unfertilized blastodisc, however, are in various stages of degeneration (Lécaillon, 1910a; Bartelmez and Riddle, 1924; Kosin, 1945), and a large proportion of the mitotic figures are abnormal (Lécaillon, 1910a). The fact that the diploid number of chromosomes appears to be present is due, no doubt, to the failure of the second polar body to be extruded when fertilization does not occur (Kosin, 1945).

Kosin (1945) remarked that practically all nuclei disappear within 24 hours after the egg is laid. Lécaillon (1910a) found them still present, although markedly degenerated, as much as a week afterward; but when he subjected the new-laid egg to incubation temperature, the nuclei vanished within 24 hours. He found no mitotic figures in eggs held at room temperature for more than 1 day.

It appears, therefore, that the infertile avian egg may be capable of segmentation resembling true cleavage, but that degeneration of the blastodisc begins prior to the time the egg is laid. Incubation does not stimulate segmentation; on the contrary, it accelerates the degenerative process.

## THE FORMATION OF PRIMARY ENDODERM

While the blastoderm is still concavo-convex, a distinct lower layer appears. This lower layer, the primary endoderm, or hypoblast, is separated from the upper layer by a narrow fissure and from the yolk by a wider space. The hypoblast soon extends throughout the entire area pellucida and into the region of the germ wall.

The question of how the primary endoderm originates has been under discussion for a number of decades. Many observers have noted that the hypoblast is found in the posterior part of the area pellucida of the very early blastoderm and that the hypoblastic cells of this region eventually join other hypoblastic cells which grow forth from the germ wall around the entire circumference of the area pellucida. It was logical, therefore, for

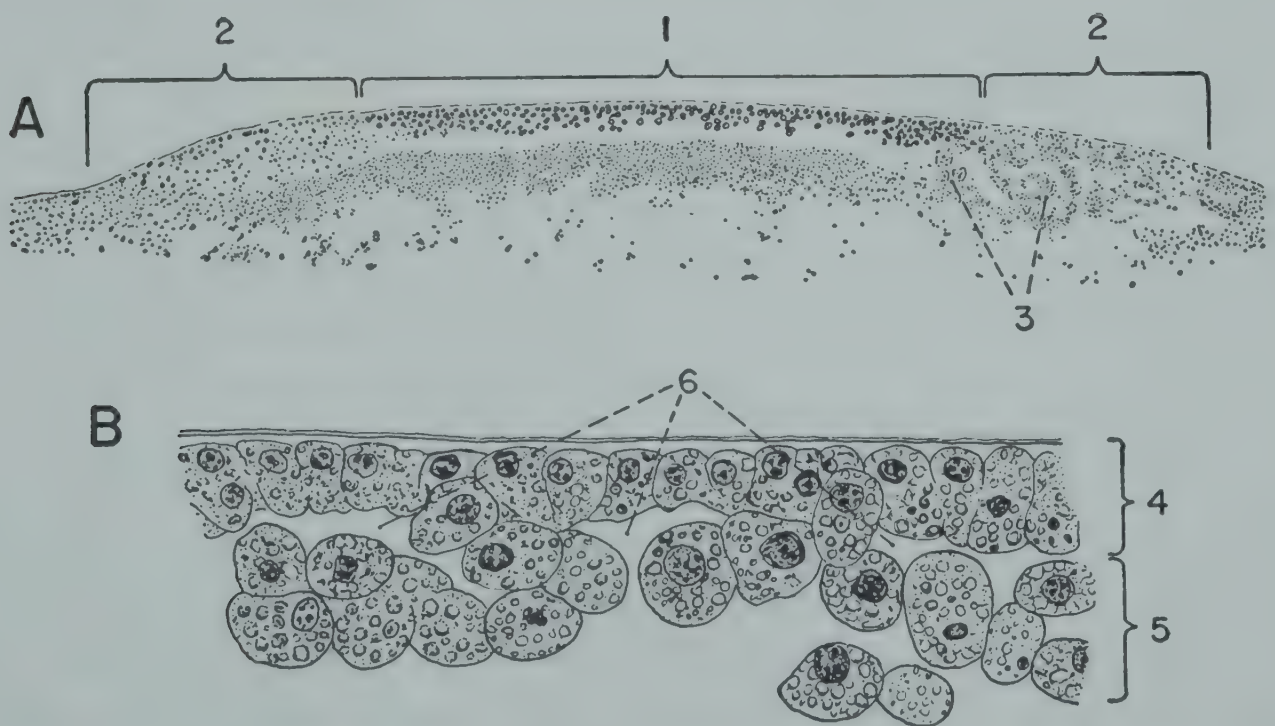


various embryologists to conclude that the primary endoderm originates by the invagination of cells located either around the whole periphery of the blastoderm (Goette, 1874; Disse, 1878) or preponderantly at its posterior margin (Duval, 1884a; Patterson, 1909b). There is, however, an alternate view, first implied by Pander (1817, *pp.* 26–27), who stated that the inner germ layer is derived from the outer. Subsequently, numerous workers agreed that the primary endoderm is a direct product of the ectoderm (epiblast or ectoblast), its cellular elements undergoing differentiation as they delaminate from the latter (Baer, 1828b; Remak, 1855; His, 1868, *p.* 61; Oellacher, 1872; Balfour, 1873a; Rauber, 1876b; Kölliker, 1879, *p.* 84; Kionka, 1894; Mitrophanow, 1899, 1901, 1902; Schauinsland, 1899). Experimental evidence continues to favor this opinion (Peter, 1938b, 1938c; Pasteels, 1945; Spratt, 1946).

In addition to the two major theories of endoderm formation—that is, the delamination theory and the invagination theory—a few other hypotheses have been proposed but have never received much support.

### The Delamination Theory

A detailed account of endoderm formation by delamination is found in two papers by Peter (1938b, 1938c). According to this author's description, the primary endoderm forms almost imperceptibly, and its origin can be traced back to the stage when the biconvex blastoderm first begins to thin



**Fig. 48.** The early formation of hypoblast. (Redrawn with modifications after Peter, 1938c.)

A, a sagittal section through the blastoderm of a pigeon's (*Columba livia*) egg about 37 hours after fertilization ( $\times 40$ ); B, an enlarged portion of the section shown in A, revealing the alignment of the superficial cells and the fissures separating them from the deeper cells ( $\times 300$ ).

1, area pellucida; 2, area opaca; 3, yolk spheres; 4, epiblast; 5, hypoblast; 6, fissures between epiblast and hypoblast.



out slightly in the center. At this time, it is occasionally possible to discern small horizontal slits or fissures appearing sporadically between the uppermost layer of cells and the larger elements underneath. After the blastoderm becomes concavo-convex, it still appears in cross section as an uninterrupted mass of cells, but the clefts are somewhat more apparent (Fig. 48-A). The superficial cells, still more or less round in shape, soon begin to align themselves fairly regularly throughout the area pellucida (Fig. 48-B). The underlying cells are scarcer anteriorly, where the area pellucida is thinnest.

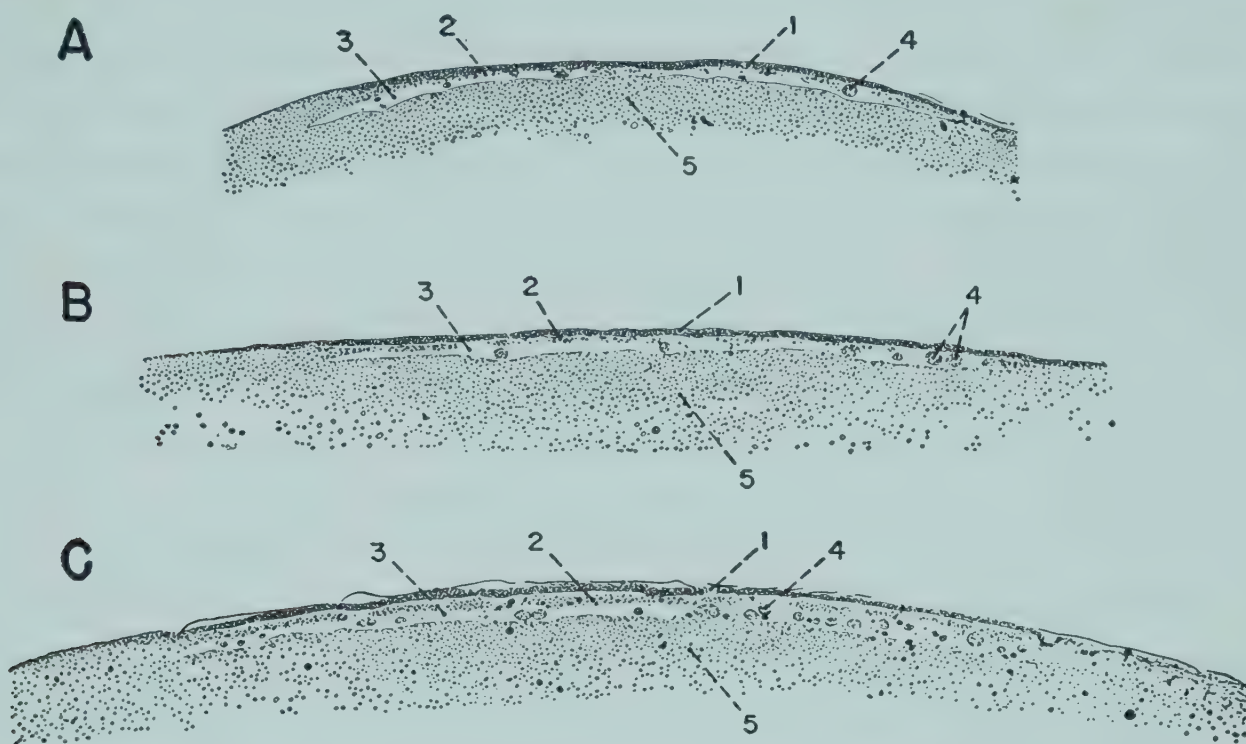


Fig. 49. Successive stages in the formation of the hypoblast in the chicken (*Gallus gallus*) blastoderm. (Redrawn with modifications after Peter 1938b.)

A and B, stages prior to incubation; C, development attained a few hours after the beginning of incubation. The posterior end of the sections is at the left. All  $\times 23$ .

1, epiblast; 2, hypoblast; 3, subgerminal cavity; 4, yolk spheres in subgerminal cavity; 5, yolk.

The blastoderm continues to grow thinner, so that the nucleus of Pander begins to shine through the area pellucida (Fig. 47-C) as a vague light spot (Duval, 1884a; Peter, 1938b). The cells at the surface of the entire area pellucida now become compressed together into a recognizable cuboidal epithelium, whereas the deeper elements retain their round form. The horizontal clefts gradually increase in size and number. In scattered areas where no fissures are present, the cells are of the same type throughout the depth of the blastoderm, and mitotic spindles lie perpendicular to the surface (Peter, 1938b). The germ wall is not yet divided into layers. It is somewhat thicker posteriorly, where the subgerminal cavity has started to undermine it (Fig. 49-A). The deeper cells of the posterior germ wall are continuous with those forming the lower stratum of the area pellucida. The cells of this stratum are disposed in two layers posteriorly but are even scarcer anteriorly than before. The polarity of the blastoderm is growing more apparent.



The blastoderms of the duck, *Anas platyrhynchos* (Pasteels, 1945), the pigeon, *Columba livia* (Peter, 1938c), and the turkey, *Meleagris gallopavo* (Kosin, 1951), are in this stage of development approximately at the time the egg is laid. There is no single appearance of the blastoderm that can be described as typical of the new-laid chicken egg, in which endoderm development may or may not have advanced somewhat farther. The differentiation of the endoderm apparently proceeds at a rate characteristic of eggs of the individual hen (Hays and Nicolaides, 1934).

The splitting of the blastoderm into two layers grows increasingly apparent as the horizontal fissures coalesce (Fig. 49-B). Within a short time, there is an almost complete separation of the upper layer from the lower. The differentiation of the two primary germ layers gradually extends into the region of the germ wall throughout the entire circumference of the blastoderm. At the periphery of the blastoderm, the germ wall ectoderm begins to progress over the yolk. The subgerminal cavity has continued to penetrate beneath the germ wall, whose lower stratum is now composed of endoderm. The uninterrupted ring of endoderm encircling the area pellucida is wider caudally, since the endoderm extends forward from the posterior germ wall for a short distance as a continuous lamina two cells or more in thickness. More anteriorly, the endoderm is broken up by many small lacunae and becomes a lacy but still coherent layer. This gradually thins out, so that only a very few endodermal cells are scattered singly in the cranial half of the area pellucida.

In general, the most advanced blastoderms of new-laid chicken eggs have progressed no farther than this stage. All subsequent development takes place after the beginning of incubation.

The blastoderm of the unincubated chicken egg has been reported to measure anywhere from 2.5 to 5.8 mm. in diameter (His, 1868; Duval, 1889; Dareste, 1891, p. 287; Assheton, 1896; Foster and Balfour, 1893, p. 4; Mitrophanow, 1899; Edwards, 1902; Dehnel, 1929; Butler, 1935; Romanoff, Smith, and Sullivan, 1938; Funk and Biellier, 1944; Spratt, 1946). The average diameter is usually from 3.0 mm. (Dehnel, 1929) to 3.5 mm. (Mitrophanow, 1899), sometimes as much as 3.7 (Butler, 1935) to 4.4 mm. (Edwards, 1902). The width of the area pellucida may range from 1.5 to 2.7 mm. (Butler, 1935), but averages about 2.0 to 2.5 mm. (Edwards, 1902; Dehnel, 1929; Butler, 1935).

The diameter of the unincubated blastoderm of the duck (*Anas platyrhynchos*) averages about 2.2 mm. (Chen, 1932) but may range from 1.8 mm. (Chen, 1932) to close to 3.0 mm. (Mitrophanow, 1902). The area pellucida varies from 0.8 to 1.5 mm. in diameter, with an average of 1.1 mm. (Chen, 1932). Although the duck's egg is larger than the chicken's, its blastoderm is slightly smaller. The unincubated blastoderm of the tern



(*Sterna hirundo*) is more comparable in size to the chicken's, since it measures from 3.5 to 4.0 mm. in diameter (Mitrophanow, 1902).

Mitrophanow (1901) described the blastoderm of the rook (*Corvus frugilegus*) as measuring 1.5 to 1.8 mm. in diameter before hypoblast formation is completed.

After 6 hours of incubation, there are visible changes in both the epiblast and the hypoblast of the chick blastoderm. Centrally and posteriorly in the area pellucida, the epiblast cells have grown tall. The hypoblast cells have flattened out in the posterior region. In the loosely knit intermediate zone of the hypoblast, the interstices begin to be filled with cells, so that the hindermost, continuous stratum may be considered to have extended forward. Soon the hypoblast becomes an uninterrupted sheet everywhere (Fig. 49-C) except in a narrow semicircular area, immediately inside the anterior germ wall, where it remains lacking entirely (Peter, 1938*b*, 1938*c*). This area will be filled with hypoblast derived from the germ wall, but not until the primitive streak appears.

### The Invagination Theory

Goette (1874) suggested that the involution or inrolling of cells around the margin of the avian blastoderm forms the germ wall, from which the hypoblast grows centripetally. It was the posterior rim of the blastoderm, however, that was designated as the site of endoderm invagination by Duval (1884*a*) and Patterson (1909*b*), the principal sponsors of the invagination theory.

Duval stated that the blastoderm is separated from the yolk posteriorly by a slitlike subgerminal cavity, which at first extends only a short distance anteriorly but which gradually burrows forward under the entire blastoderm. Kionka (1894), however, felt that the cleft beneath the posterior germ wall in Duval's sections was an artifact; O. Hertwig (1906, *p.* 861) also expressed this opinion, although at one time he supported Duval's theory (Hertwig, 1892, *pp.* 94-97).

The account of primary endoderm formation given by Patterson (1909*b*) has been read and quoted so widely that it must be reviewed here. Fundamentally, Patterson was in agreement with Duval, but he pursued the question in minute detail, adding his own interpretations of the phenomena he observed.

Patterson indicated that endoderm formation progresses very rapidly in the pigeon's (*Columba livia*) egg, beginning at about the thirty-third or thirty-fourth hour after fertilization, or 7 to 9 hours before the egg is laid. Soon after the central part of the blastoderm has thinned out to give rise to the area pellucida, a small section of the posterior margin of the blastoderm



also becomes greatly reduced in thickness, and tapers off peripherally to a depth of one cell. The anterior part of the blastoderm is now the thicker, having taken on much of the former aspect of the posterior region. In fact, Peter (1938c) considered it obvious, from Patterson's illustrations, that the latter's sections were inadvertently reversed.

Beneath the attenuated portion of the posterior margin, the periblastic nuclei degenerate, so that the syncytium disappears. For a short distance, therefore, the zone of junction is interrupted; that is, the margin of the blastoderm is set free from the yolk below. Patterson's description of this occurrence raised a second objection from Peter (1938c), who, like Disse (1878) and Gasser (1882), stated that the cells of the blastoderm are always sharply set off from the yolk, except in very early stages. Peter called attention to the fact that careful inspection reveals a fine line of division separating the blastoderm from the yolk in several of Patterson's photographs. Jacobson (1938a) also stated that the nuclei in this region are not "free," for staining for glycogen makes the form of the cells appear clearly. Chen (1932), on the other hand, remarked that the entire marginal region of the duck's (*Anas platyrhynchos*) blastoderm is syncytial during this stage, not merely the portion in immediate contact with the yolk.

The interruption in the zone of junction widens rapidly and soon occupies about 70° to 80° of the blastoderm's circumference at the thirty-third hour after fertilization. The free margin of the blastoderm now rolls under. The invaginated cells, which grow forward under the epiblast, constitute the primary endoderm. A narrow space separates the redoubled posterior rim of the blastoderm from the yolk; this space is continuous with the subgerminal cavity and represents the archenteron, or primitive gut cavity.

Patterson then observed that the hypoblast grows forward as a tongue-like process with a free anterior margin, and that the germ wall, formerly present only anteriorly, gradually extends around the whole circumference of the blastoderm. (Patterson defined the germ wall as only the inner margin of the zone of junction, that is, the region where new cells are added centrally to the blastoderm.) At either side of the area pellucida, the germ wall cells begin to extend into the subgerminal cavity. Patterson stated, however, that this ingrowth of cells is only apparent and is actually due to a widening of the cavity. The invaginated hypoblast unites with these cells, which constitute "the under loose layer of the area opaca." The hypoblast thus appears to be originating from the germ wall, although, in his opinion, it does not really do so.

As the blastoderm increases in area, the zone of junction spreads out centrifugally. It forms "horns" which grow out on either side of the posterior gap and approach each other. Eventually, the horns meet, the gap is obliterated, and the zone of junction once more encircles the blastoderm



completely. In this way, the site of endoderm formation is brought to the posterior edge of the area pellucida.

A number of investigators failed to find clear-cut evidence of endoderm formation by invagination in blastoderms of the chicken (*Peter, 1938b; Spratt, 1946*), the duck, *Anas platyrhynchos* (*Chen, 1932; Pasteels, 1945*), and the pigeon, *Columba livia* (*Pasteels, 1937*). Pasteels (1937) alone noted the occasional presence of a posterior rounded swelling resembling the structure described by Patterson, but he attributed it to retarded extension of the blastoderm due in turn to unequal segmentation. He detected nothing that could be interpreted as an inrolling of cells.

As Peter (1938c) pointed out, the deeper-lying cells are larger than the superficial ones and therefore cannot be derived from the latter simply by the doubling of the epiblast upon itself. Again, Peter (1938c) criticized Patterson's failure to recognize the cells of "the under loose layer of the area opaca" as true hypoblast cells girdling the periphery of the area pellucida. It also seems possible that Patterson erred in his assumption that the hypoblast pushes itself forward under the epiblast, since Peter (1938b, 1938c) found evidence that the two layers expand together. Patterson's description of the later stages of primary endoderm formation therefore corresponds in many morphological details with that given by Peter (1938b, 1938c), but his interpretation diverges. According to Assheton (1912), Patterson's theory is inadequately supported by his experimental findings and is compatible with the embryogeny of fish rather than with that of reptiles and mammals, to which birds are more closely related.

It is of considerable interest, however, to mention the observations of Lutz and Reyrolles (1952) and Lutz (1953, 1955), who placed carbon particles on the surface of the duck's (*Anas platyrhynchos*) blastoderm before incubation and noted the subsequent displacements and changes in shape of the marks. The results seemed to indicate a centrifugal movement of superficial cells and their invagination into a groove around the periphery of the blastoderm. According to these authors, the process of invagination begins at the posterior border of the blastoderm; elsewhere, it is of a secondary nature. The endodermal cells originating posteriorly grow forward and join the cells invaginated around the anterior and lateral borders.

### Other Theories

The literature contains many references to the fact that the hypoblast apparently grows forward from the posterior germ wall, with which it is continuous, the implication being that the germ wall gives rise to the hypoblast as a proliferation of cells. Nowack (1902) tentatively suggested this origin, while indicating that hypoblast cells may also detach themselves from the epiblast. Chen (1932) found that the majority of mitotic figures in the hypoblast are oriented in an anteroposterior direction and therefore



concluded that the hypoblast migrates forward from the posterior germ wall in complete independence of the epiblast. Against this theory may be placed the observation that epiblast and hypoblast grow concomitantly (*Peter, 1938b, 1938c*).

Another suggestion has been termed the polyinvagination theory. Gräper (1929) reported that the area pellucida of vital-stained blastoderms presents a wrinkled, crepy appearance. It was supposedly demonstrated by Merbach (1935) that this appearance is due to the presence of multiple, small plications. Sections seemingly showed that the epiblast and hypoblast are continuous with each other at the bottom of every fold and that individual cells apparently migrate out of the upper germ layer into the lower. The observed plications disappeared upon subsequent incubation and were replaced by a transitory marginal furrow persisting longest posteriorly. Merbach suggested that the posterior groove is identical with the "sickle" seen by Koller (1879, 1882) but never fully explained. Peter (1938*b*), however, dismissed both Merbach's and Koller's findings as artifacts and described the manner in which they may be produced at will.

Pasteels (1937) also subscribed temporarily to the polyinvagination theory because he noted many flask-shaped cells which, in the later stages of primary endoderm formation, appeared to be migrating out of the superficial layer to form the underlying stratum. Later (*Pasteels, 1940*), he repudiated his former belief, since he found that the flask cells are not demonstrable when the hypoblast begins to differentiate. As an alternative, he suggested the possibility that these cells are migrating into the superficial layer and thus contributing to the expansion of the epiblast (*Pasteels, 1945*).

Jacobson (1938*a*) proposed yet another mode of origin for the primary endoderm. He stated that the epiblast of the unincubated chick blastoderm becomes extended as a single-layered epithelium, the cells of which are taller posteriorly. In the thicker posterior region, single epiblast cells migrate downward to become hypoblast cells, which soon start to move anteriorly, singly or in small groups. The entire area of invagination eventually bends inward to form an archenteric canal, the lumen of which shortly breaks through into the subgerminal cavity. Pasteels (1945) criticized Jacobson strongly, inasmuch as the latter's photographs suggested that the phenomena which he observed were largely artifacts. Spratt (1946), furthermore, stated that canals resembling the "archenteron" described by Jacobson can be produced by technical manipulation.

### The Identification of Fertile and Infertile Unincubated Eggs

Since embryonic development has progressed at least to the early stages of endoderm formation by the time the egg is laid, the cellular nature of the blastoderm serves to identify a fertilized but unincubated egg. The



presence of cells can be revealed by staining the blastoderm *in situ* on the yolk, if the latter is hardened in formaldehyde (Kosin, 1944b).

An infertile blastodisc can be recognized macroscopically or at low magnification because dark vacuoles usually develop in it before the egg is laid. These vacuoles were noted by Prevost and Dumas (1827), Coste (1847), His (1868), Kosin (1944b), and—according to Dareste (1864)—by Malpighi in the seventeenth century. They are characteristic of infertile eggs not only of the chicken but also of the turkey, *Meleagris gallopavo* (Kosin, 1951), the peahen, *Pavo cristatus* (Lécaillon, 1909c, 1910a), and the ducks, *Anas platyrhynchos* and *Cairina moschata* (Chappellier, 1912a).

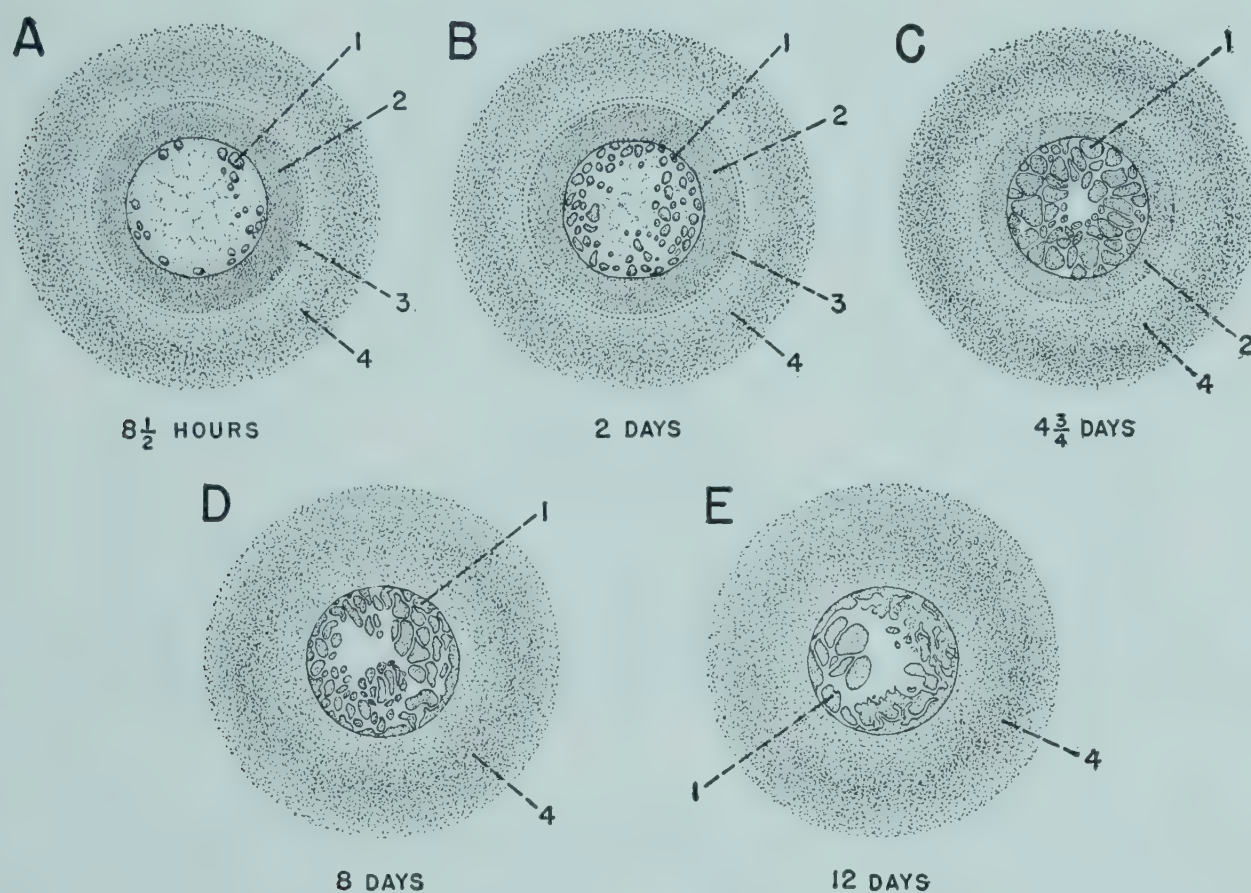


Fig. 50. The progressive disintegration of the infertile blastodisc of the chicken (*Gallus gallus*) egg held at room temperature, as seen in surface views. (Redrawn with modifications after Lécaillon, 1910a.) All  $\times 5$ .

1, vacuole; 2, yellow ring; 3, white ring; 4, yolk.

In cross section, the vacuoles appear to be filled with fluid (Kosin, 1951). They are sometimes present in fertilized eggs but are usually confined to the area opaca of the blastoderm.

With the passage of time, the vacuolated zone in the infertile blastodisc widens at the expense of the central region, although never obliterating the latter entirely (Lécaillon, 1910a). Eventually, there is a confluence of the vacuoles and disintegration of the network of substance separating them. Figure 50 shows the appearance of the blastodisc in chicken eggs held at ordinary temperatures for 8.5 hours and for 2, 4.75, 8, and 12 days.

The average high-frequency conductivity of groups of new-laid fertile eggs differs from that of groups of new-laid infertile eggs.

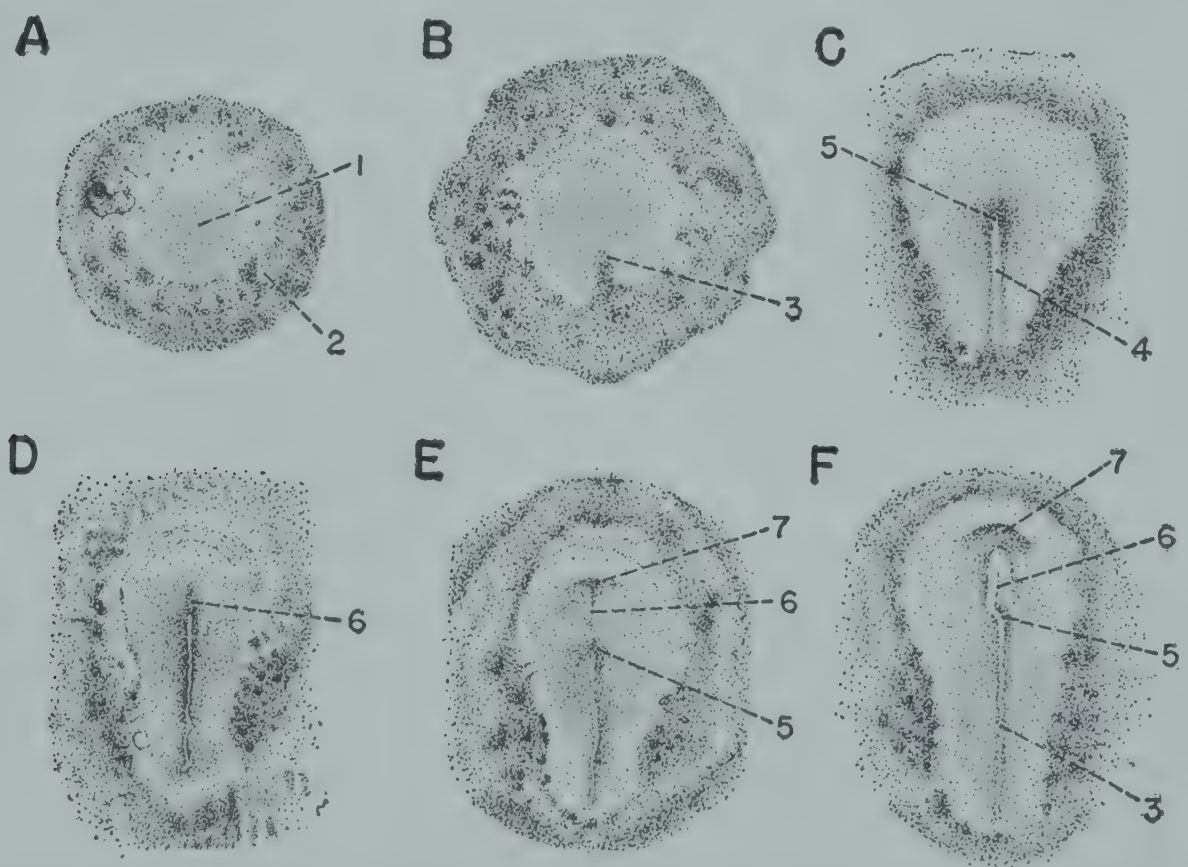


## THE PRIMITIVE STREAK AND THE FORMATION OF THE MESOBLAST

Since the early days of embryology, it has been known that the immediate forerunner of the avian embryo is a linear thickening that appears in the posterior portion of the area pellucida. Because of its shape, this structure has long been known as the primitive streak (*Baer, 1828b*). Very shortly after the formation of the primitive streak, a third primary germ layer—the mesoblast or mesoderm—can be seen between the epiblast and the hypoblast, migrating outward from the streak.

### The Pre-Streak Blastoderm

In the hours immediately preceding the formation of the primitive streak, there is little change in the appearance of the blastoderm, except for the development of the so-called “embryonic shield” (*Baer, 1828b; Balfour and Deighton, 1882; Koller, 1882; Nowack, 1902; Spratt, 1946*) in the central



**Fig. 51.** Several successive stages in the development of the avian blastoderm, as seen in surface views showing the embryonic shield and the primitive streak of various lengths. (Redrawn with modifications A, B, after Spratt, 1946; C, D, E, after Chen, 1932; F, after Wetzel, 1929.)

A, the chicken (*Gallus gallus*) blastoderm shortly before the appearance of the primitive streak, in the stage of the embryonic shield; B, the short primitive streak in the chicken blastoderm; C, the definitive primitive streak in the duck (*Anas platyrhynchos*) blastoderm; D, the early head-process stage in the duck blastoderm; E, the late head-process stage in the duck blastoderm, with the medullary plate thickened anteriorly; F, the early head fold stage in the chicken blastoderm. All  $\times 8$ .

1, area pellucida; 2, area opaca; 3, primitive streak; 4, primitive groove; 5, region of the primitive node; 6, head-process; 7, head fold.



posterior part of the area pellucida. The shield is a relatively opaque, circular region, densest medially and thinning out to transparency peripherally (Fig. 51-A). According to Nowack (1902), it is largely a manifestation of the varying thickness of the epiblast, the cells of which have attained their greatest height and become pseudostratified slightly behind the center of the area pellucida (Fig. 52-A). Waldeyer (1869) and Koller (1882), how-

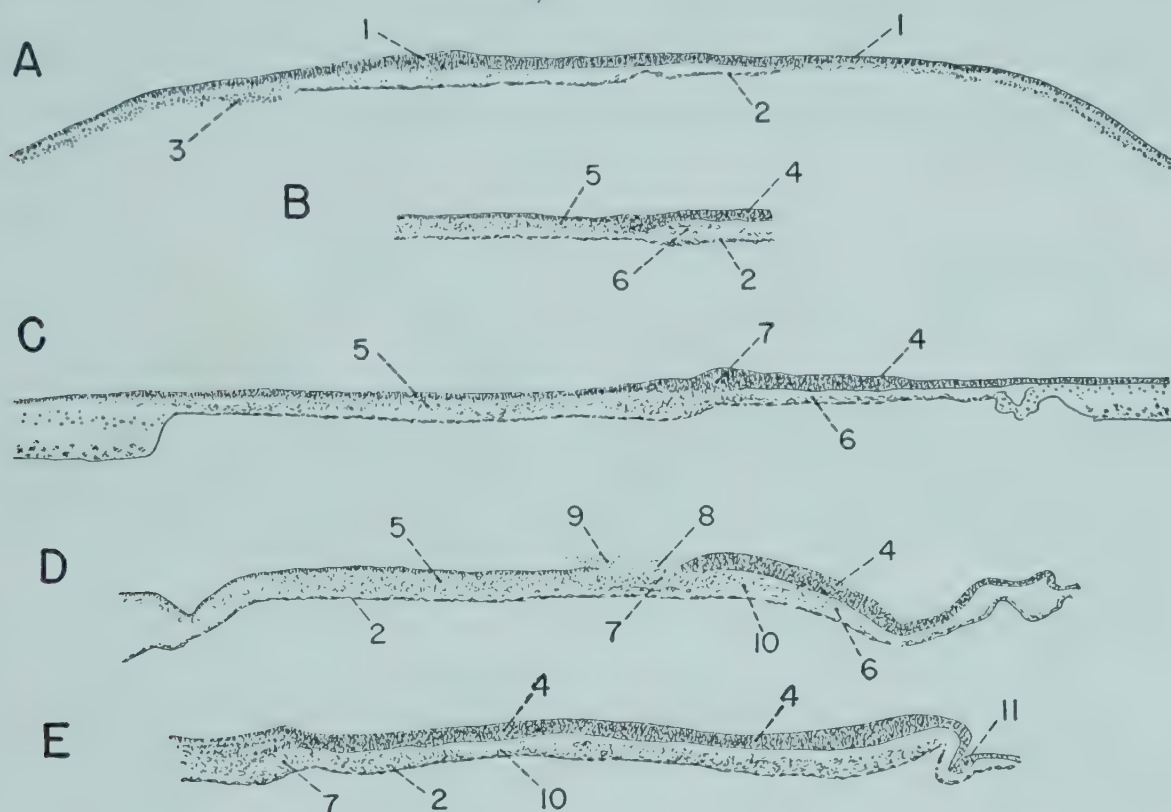


Fig. 52. The development of the avian blastoderm from the stage immediately preceding the appearance of the primitive streak to that of the formation of the head fold. (Redrawn with modifications A, D, after Chen, 1932; B, after Spratt, 1946; C, E, after Duval, 1889.)

A, pre-streak or embryonic shield stage of the duck (*Anas platyrhynchos*) blastoderm, median sagittal section; B, median broad primitive streak stage (anterior end of streak) of the chick (*Gallus gallus*) blastoderm, same section; C, definitive primitive streak stage of the chick blastoderm, same section; D, late head-process stage of the duck blastoderm, same section; E, early head fold stage in the duck, same section. All  $\times 35$ .

1, epiblast; 2, hypoblast; 3, germ wall; 4, medullary plate; 5, primitive streak; 6, pre-axial mesoderm; 7, primitive node; 8, primitive pit; 9, primitive fold; 10, head-process or notochord; 11, head fold.

ever, attributed the presence of the shield principally to the multilayered condition of the hypoblast in the caudal half of the pellucid area. Chen (1932) and Spratt (1942, 1946) considered changes in both epiblast and hypoblast to be responsible. Other workers (Mitrophanow, 1899; Dehnel, 1929; Wetzel, 1929) suggested that the arrangement of white yolk and the depth of the subgerminal cavity contribute to this formation.

### The Short Streak

In the hen's egg, the primitive streak is first visible at about the eighth or ninth hour of incubation, when the diameter of the blastoderm is 4.5 to 6.0 mm. (Mitrophanow, 1899) and the diameter of the area pellucida is



2.0 mm. (Wetzel, 1929) to 2.3 to 3.0 mm. (Mitrophanow, 1899). In the blastoderms of other birds, its time of appearance is different, of course; thus it arises at some time between the ninth or tenth (Mitrophanow, 1902) and the twelfth or fiftieth hour (Chen, 1932) in the duck's (*Anas platyrhynchos*) blastoderm—diameter 3.4 mm. (Chen, 1932)—and at approximately the fiftieth in that of the emu, *Dromaeus novae-hollandiae* (Haswell, 1887). The blastoderm of the tern (*Sterna hirundo*) measures about 4.0 mm. in diameter when the streak appears (Mitrophanow, 1902), and that of the rook (*Corvus frugilegus*) about 2.0 by 2.5 mm. (Mitrophanow, 1901).

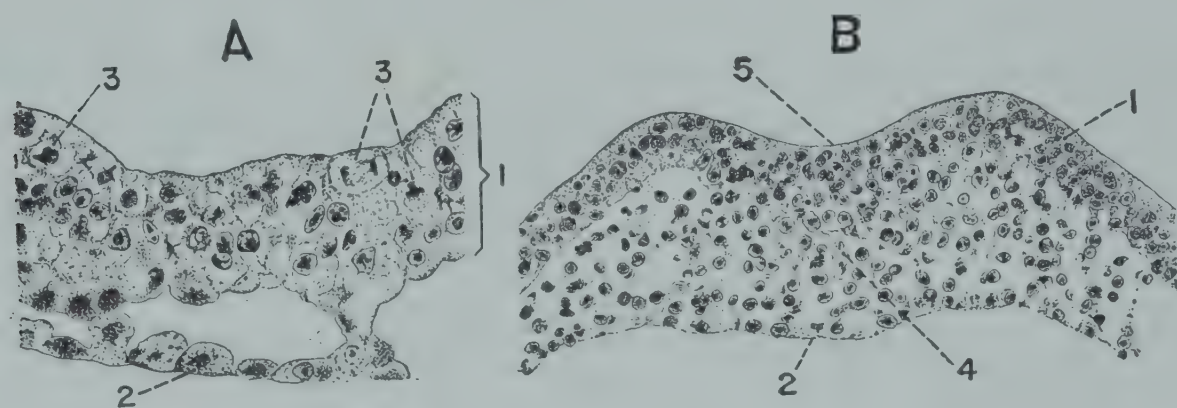


Fig. 53. Two stages in the development of the primitive streak, as seen in cross sections of the streak. (Redrawn with modifications A, after Wetzel, 1929; B, after Wetzel, 1932.)

A, section showing thickening of the epiblast in the midline and mitoses of epiblast cells ( $\times 300$ ); B, section through the anterior portion of the medium to long streak of the pyriform blastoderm, showing the primitive groove ( $\times 150$ ).

1, epiblast; 2, hypoblast; 3, mitoses in epiblast; 4, primitive streak; 5, primitive groove.

The first indication of primitive streak formation is seen as a downward movement of cells in ectoderm that is thickened along a line bisecting the posterior portion of the area pellucida. In the beginning, this line occupies only about one fifth to one quarter (Wetzel, 1929) or one third (Spratt, 1946) of the longitudinal axis of the pellucid area. Jacobson (1938b), however, claimed that the inception of the streak can be detected at a stage when much less than one fifth of the axis is affected. Mitrophanow (1899) stated that streak formation starts centrally in the chick blastoderm and proceeds posteriorly toward the germ wall; a similar mode of origin has allegedly been seen in blastoderms of the sparrow, *Passer domesticus* (Schauinsland, 1903a), the duck, *Anas platyrhynchos* (Mitrophanow, 1902), and the rook, *Corvus frugilegus* (Mitrophanow, 1902). According to Wetzel (1929), the ectoderm becomes several cell layers deep along the entire length of the incipient streak, but the thickened zone is deepest and widest anteriorly and posteriorly and even extends into the posterior germ wall. Wetzel believed that streak formation is initiated by the lo-

calized proliferation of cells (Fig. 53-A), but others have felt that the invagination of epiblast is alone responsible (*Pasteels, 1937; Jacobson, 1938b*).

As Waldeyer (1869) remarked, the earliest visible streak is broad and of indefinite outline. In surface view, it is seen as a cone-shaped region of increased density projecting forward from the posterior germ wall (Fig. 54-A). The early primitive streak of the chick is about 0.4 mm. long (*Spratt, 1942*); that of the duck (*Anas platyrhynchos*) averages 0.32 mm. in length (*Chen, 1932*). As soon as it appears, the streak begins to grow

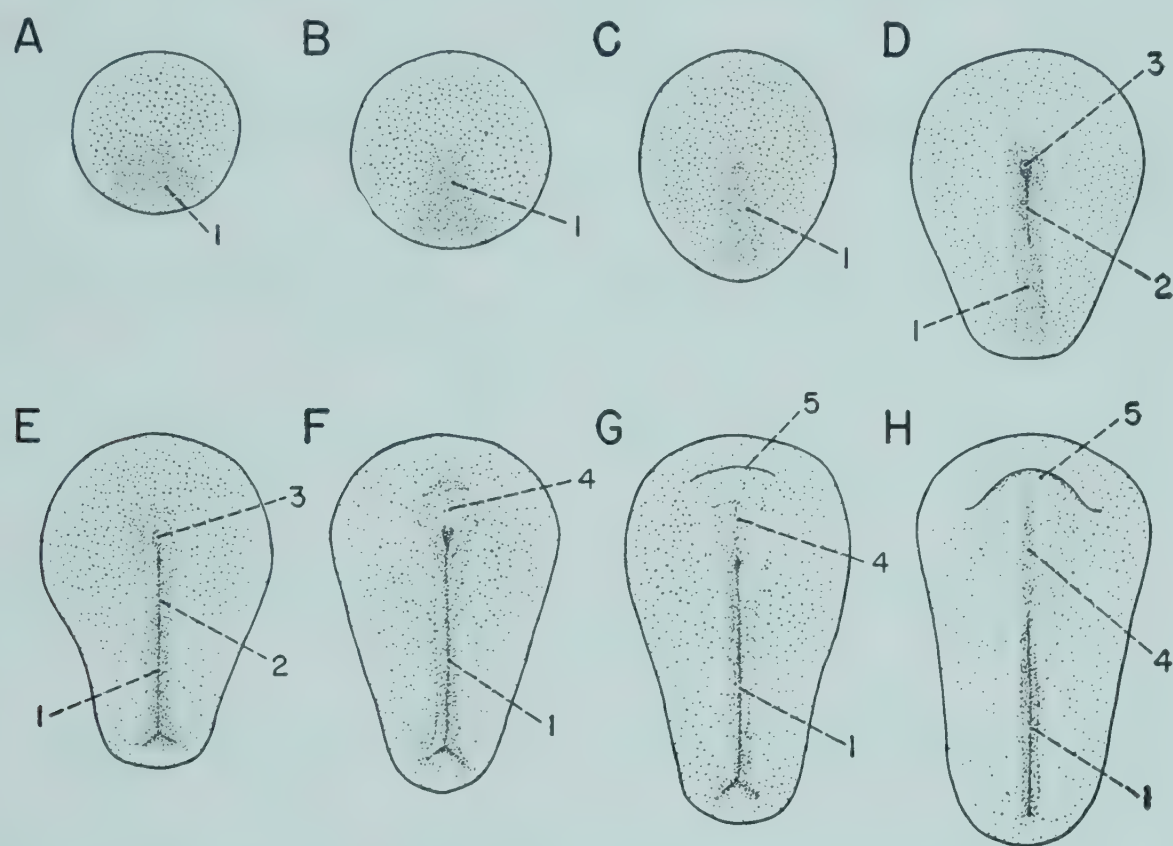


Fig. 54. Successive stages in the development of the primitive streak of the chick (*Gallus gallus*) from its inception to the head fold stage, shown in semidiagrammatic surface views of the area pellucida. (Redrawn after Dalton, 1935.)

A, 8 hours; B, 10 hours; C, 12 hours; D, 14 hours; E, 16 hours; F, 17 hours; G, 18 hours; H, 19 hours of incubation.

1, primitive streak; 2, primitive groove; 3, primitive pit; 4, head-process; 5, head fold.

and to become narrower and more sharply demarcated. In the chick blastoderm, the primitive streak changes considerably in size and shape within the first 2 hours after its formation (Fig. 51-B).

The growth and development of the streak are accompanied by changes in the structure of the blastoderm. The hypoblast, although still discontinuous anteriorly, becomes a single-layered sheet of flat, spindle-shaped cells, except in a localized, horseshoe-shaped region that arches around the forward end of the streak. Here the hypoblast is several layers thick (*Wetzel, 1929; Peter, 1938b*), although clearly separated from the epiblast above it (Fig. 55-A<sub>1</sub>). Carbon-marking experiments (*Fraser, 1954*) show that



migration of cells from the epiblast into the lower germ layer is still taking place in this region and at the anterior and posterior borders of the area pellucida. The hypoblast is spreading centrifugally from the anterior end of the early streak, progressing more rapidly in the cephalad direction (*Fraser, 1954*). Later, the hypoblast becomes more or less fused with the material of the primitive streak throughout the anterior and posterior thirds of the latter, but remains free throughout the middle one third (Fig. 55-A<sub>2</sub>).

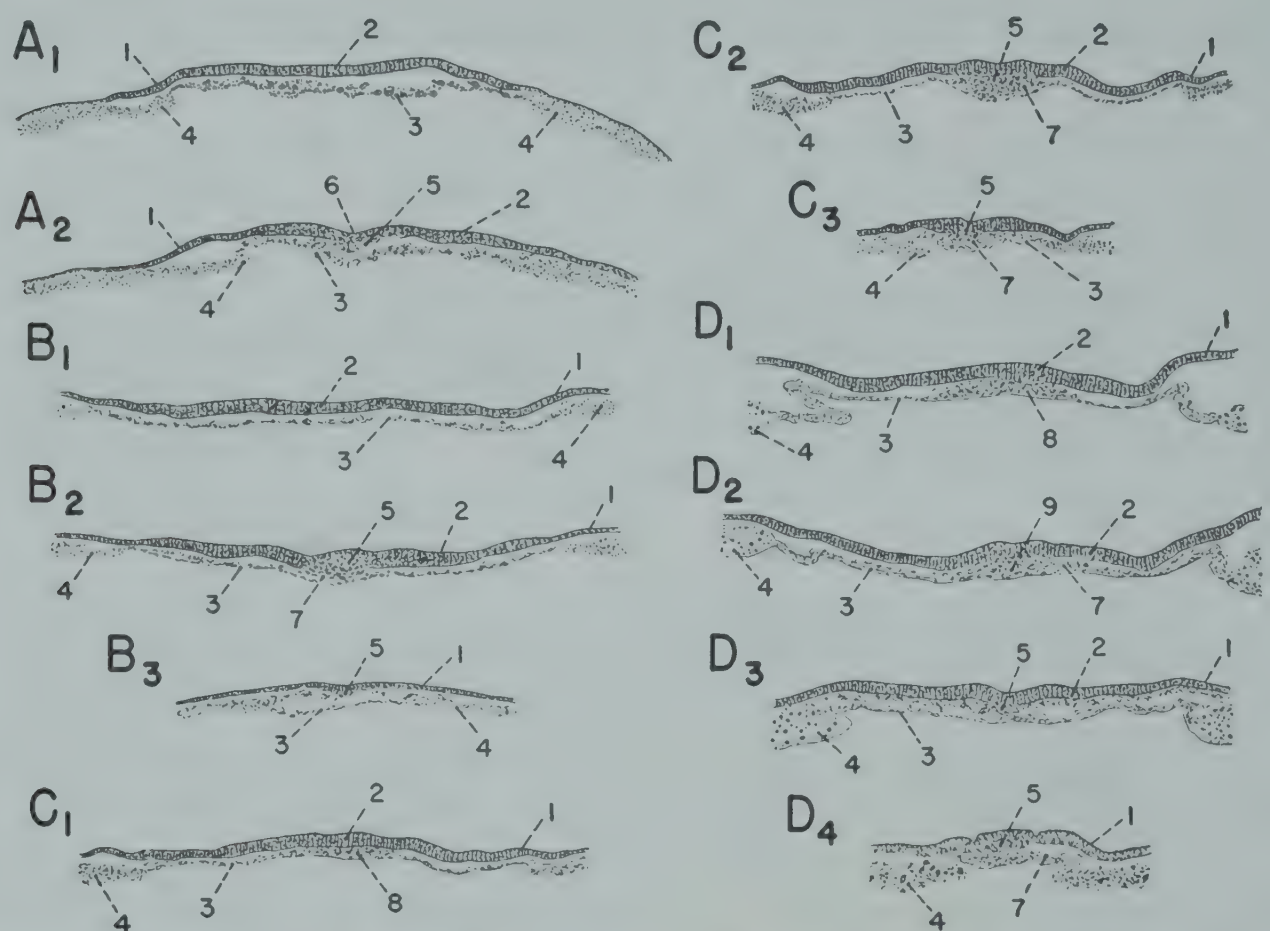


Fig. 55. The development of the primitive streak, as seen in cross sections of the duck (*Anas platyrhynchos*) blastoderm, taken at several different levels and stages. (Redrawn with modifications after *Chen, 1932*.)

A<sub>1</sub>, A<sub>2</sub>, sections taken, respectively, anterior to and through the midlevel of the short primitive streak of a 12-hour blastoderm; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, sections taken, respectively, anterior to the medium primitive streak of a 17-hour blastoderm and through its anterior and posterior ends; C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, sections taken at the same levels of the long primitive streak of a 20-hour blastoderm; D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, sections taken, respectively, anterior to the definitive primitive streak of a 23-hour blastoderm, through the node, through the mid-level of the streak, and through its posterior end. All  $\times 28$ .

1, epiblast; 2, medullary plate; 3, hypoblast; 4, germ wall; 5, primitive streak; 6, primitive groove; 7, mesoblast; 8, preaxial mesoblast; 9, primitive node.

When the streak extends across almost one third of the area pellucida, a few cells may be seen migrating forward from the free end of the streak, between hypoblast and epiblast (Fig. 52-B). A similar cellular migration, directed laterally, then begins along the length of the streak. These free cells proceeding outward from the streak are the first sparse elements of the mesoblast.

*The Axis of Symmetry*

With the formation of the primitive streak, the bilaterality of the blastoderm is at last clearly defined. Furthermore, the position and the polarity of the embryo are indicated, for the long axis of the embryonic body will coincide with that of the streak, and the head of the embryo will form at the end of the streak closest to the center of the blastoderm.

The first recorded observation on the orientation of the embryo was made by Pander (1817), who remarked that the anteroposterior axis of the embryo lay along the short diameter of the egg. A few years later, Baer (1828*b*, *p.* 12) confirmed the general truth of this statement. He found that the embryo is normally seen with its head directed to the left when the egg is held so that its blunt end is nearest the observer. He also noted, however, that the embryonic axis is not always exactly perpendicular to the egg's axis. A great many investigators subsequently attempted to determine the frequency with which the two axes meet at a right angle. The tabulation gives some typical results, in terms of the percentage of chicken eggs in which

Normally Oriented Embryos (per cent)	Investigator
50	Dalton (1881); Butler (1935)
76	Duval (1884 <i>a</i> )
9	Rabaud (1908)
32	Kopsch (1927 <i>a</i> ); Cavers and Hutt (1934)

a 90° angle was found. In view of the variations reported, it is not surprising that deviation of the embryo from the perpendicular by as much as 45° to the right or left is also frequently encountered, as the accompanying data indicate. Deviation of more than 45° from the perpendicular may occur in

Embryos Deviating ±45° from Perpendicular (per cent)	Investigator
76	Rabaud (1908)
62	Kopsch (1927 <i>a</i> )
74	Cavers and Hutt (1934)
44	Butler (1935)

5 per cent (Butler, 1935) to 29 per cent (Féré, 1900) of chicken eggs. Occasionally, the embryo is inverted, its head being directed to the right when the egg is held in the manner described by Baer. The number of embryonic inversions in chicken eggs has been variously reported as constituting 0.6 per cent (Duval, 1884*a*), 4.5 per cent (Cavers and Hutt, 1934),



12 per cent (*Dalton*, 1881; *Rabaud*, 1908), and 33 per cent (*Bartelmez*, 1918). The incidence of complete inversion, with the embryo lying perpendicular to the egg's axis but reversed in polarity, has also been found to vary in chicken eggs, as the table indicates.

Completely Inverted Embryos (per cent)	Investigator
1	Duval (1884a); Butler (1935)
11	Rabaud (1908)
6	Kopsch (1927a)
0.6	Cavers and Hutt (1934)
2	Lutz (1940, 1949)

In duck (*Anas platyrhynchos*) eggs, variability in the axis angle seems to be no greater than in chicken eggs. Féré (1896) reported that deviation from a 90° angle exceeded 45° in 65 per cent of the eggs of this species, but he made relatively few observations. Chen (1932), who found that the angle was exactly 90° in only 27 per cent of 174 duck eggs, noted that deviation from the perpendicular was less than 45° in 55 per cent. Lutz (1949) also observed that duck eggs agree with Baer's rule fully as well as hens' eggs.

In 90 per cent of pigeons' (*Columba livia*) eggs, however, the angle of incidence is 45°, according to Patterson (1909b). Bartelmez (1918) stated that it falls between 45° and 90° in 85 per cent of all cases, and also remarked that inversions are very rare in pigeons' eggs. This author found the axis angle to be 45° and 125°, respectively, in two sparrow (*Passer domesticus*) eggs. Haswell (1887) noted that the eggs of the emu (*Dromaeus novae-hollandiae*) comply with Baer's law and that the embryonic axis, in this species, never coincides with the long axis of the egg.

Figure 56 gives the distribution curves of chicken, duck (*Anas platyrhynchos*), and pigeon (*Columba livia*) eggs according to the angle observed between embryonic and egg axes. It will be noted that the mode on the curves for chicken and duck eggs falls at 90° and that there is a second maximum at 270°, the latter fact indicating a slight tendency for complete inversion to occur. On the other hand, the mode on the curve for pigeons' (*Columba livia*) eggs falls at 70°, and there is obviously less variability among these eggs than among those of the other two species.

As Bartelmez (1918) observed, one would normally expect a variation of at least 20° to either side of the mean; Cavers and Hutt (1934) considered 45° to either side as the normal limits of deviation. Greater deviation is probably due to irregularities in yolk formation during the final phase of growth, or to irregularities in oviducal function.

It should be noted that experiments confirm the predetermination of the

embryonic axis in the unincubated chicken or duck (*Anas platyrhynchos*) blastoderm. Scission of the blastoderm into bands parallel to the presumptive longitudinal axis often gives rise to a normally oriented embryo in each band (Morita, 1937; Lutz, 1948a, 1948b, 1949; Wolff, 1948). So, also, does transverse division of the blastoderm, or its partition into quarters, although the polarity of one embryo is sometimes reversed when these procedures are carried out—polarity, apparently, being more labile than the position of the embryonic axis (Lutz, 1949).

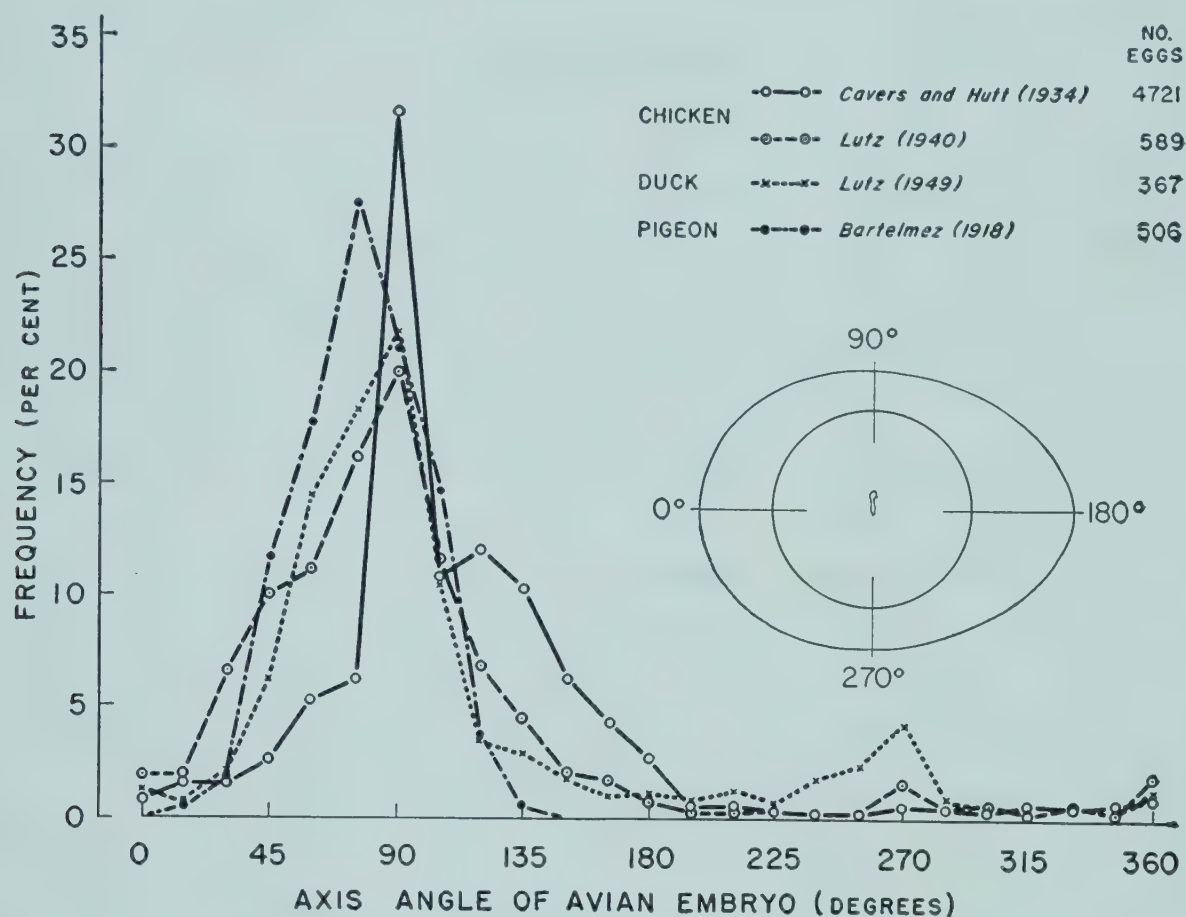


Fig. 56. The distribution of chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), and pigeon (*Columba livia*) eggs according to the angle between the long axes of the embryo and the egg. (Drawn from the data and graphs of Bartelmez, 1918; Cavers and Hutt, 1934; Lutz, 1940, 1949.)

Vintemberger and Clavert (1953) and Clavert and Vintemberger (1954) have noted a high correlation in both pigeon (*Columba livia*) and duck (*Anas platyrhynchos*) between dextral rotation during the early hours *in utero* (as indicated by the direction of winding of the chalazae) and the normal position of the embryo as described by Baer. The authors suggest that intrauterine rotation determines the anteroposterior orientation of the blastoderm.

### The Medium Streak

At first, the streak grows forward. According to Spratt (1946), it increases in length by 0.06 mm. per hour for approximately 6 hours after its inception. Elongation at this rate brings the free end of the streak to the



center of the area pellucida (Fig. 54-B) and beyond the middle of the embryonic shield. In the chick blastoderm, the streak is now about 1.2 mm. long (Wetzel, 1929); in the duck (*Anas platyrhynchos*) blastoderm, it measures about 0.6 mm. in length (Rabl, 1923; Chen, 1932). Its cranial end is broader and thicker in the region where the protuberance, known as the primitive (or Hensen's) node, will soon become prominent. It has been suggested (Spratt, 1942) that the formation of the node is the result of contact with the anterior end of the streak with cells near the center of the area pellucida that are destined to form the chorda. In the region of the node, the hypoblast is intimately fused to the overlying streak material (Fig. 55-B<sub>2</sub>), as it is also for a short distance just forward of the posterior germ wall.

A shallow depression, at first visible only in sectioned blastoderms, forms along the long axis of the anterior half of the streak before the twelfth hour of incubation (Balfour, 1873*b*); this is the incipient primitive groove. The time of appearance of the groove seems to be more variable in the duck (*Anas platyrhynchos*) than in the chick blastoderm (Chen, 1932). The mesoderm has by now grown farther out laterally along the length of the streak.

There is some difference of opinion as to the mechanism by which the streak grows to the center of the area pellucida. Balfour and Deighton (1882), Nowack (1902), Jacobson (1938*b*), and Spratt (1946) all noted that the increase in the length of the streak during this period is due in part to the addition of cells in front of the original forward end. Jacobson, in fact, remarked that the streak, in its early stages, may actually be discontinuous anteriorly. Both Wetzel (1929) and Pasteels (1937), on the other hand, believed that the streak elongates from the start through the growth of its middle portion, so that the foremost end is always the definitive anterior end, representing Hensen's node. According to this view, the streak, proceeding through the area pellucida, displaces the areas ahead of it in an anterolateral direction. On the other hand, a carbon mark placed at the free end of the early streak remains in the same spot while the streak grows out in front of it (Spratt, 1946; Nabrit, 1951). Since Wetzel (1929) and Pasteels (1937) used a vital-staining technique, the illusion that the streak was pushing forward through the area pellucida as a separate entity could have been created by the diffusion of the strains from epiblast to hypoblast, the hypoblast alone participating in the anterior movement.

After the formation of Hensen's node, it appears that no further additions are made to the forward end of the streak (Spratt, 1946). However, the growth of the streak up to this time has not been due entirely to anterior additions, for some material has been added to the posterior end as well; also, there has been a certain amount of intussusceptive growth in the posterior half of the streak. The table gives a summary (Spratt, 1946)



of the extent to which the total length of the streak up to this stage is attributable to its original length and to growth in various regions.

Region of Growth	Proportion of Total Length (per cent)
Anterior	31.8
Posterior	11.1
Intussusceptive	14.36
Original streak	42.74

### The Long Streak

After about 12 hours of incubation (Wetzel, 1929), the primitive streak of the chick has increased to a length of approximately 1.6 mm. The area pellucida has extended itself caudally (Balfour, 1873*b*; His, 1877) and now tapers slightly from anterior to posterior (Fig. 54-C); its greatest diameter measures 2.5 mm. (Wetzel, 1929). It is evident that growth has occurred chiefly in the posterior direction.

In the duck's (*Anas platyrhynchos*) egg, this stage of development has been observed at various times from the thirteenth to the twenty-fifth hour of incubation (Chen, 1932). The primitive streak of the duck is now about 0.9 mm. long, and the area pellucida averages 1.8 mm. in length.

In the pyriform blastoderm, the primitive node is not very sharply defined at first, especially in the duck's (*Anas platyrhynchos*) egg (Fig. 55-D<sub>2</sub>). The primitive groove is deepest anteriorly (Fig. 53-B) and gradually becomes wider and shallower about halfway down the streak (Fig. 54-D). In the caudal half of the streak, the hypoblast is so closely united with the "ectomesoderm" (the material of the streak) as to lose its identity. In its very hindmost portion, the streak is wide but of decreased thickness and is not in contact with the hypoblast (Fig. 55-C<sub>3</sub>). Posterior to the end of the streak, the epiblast has become lifted off the germ wall for a distance approximately coinciding with that to which the subgerminal cavity has burrowed beneath the germ wall. The detachment of the epiblast from the germ wall spreads laterally and eventually around the entire circumference of the pellucid area, with the result that a double boundary line is formed. The inner line, representing the junction of the hypoblast and the germ wall, marks off the pyriform area; the outer line indicates the limit of the loosened epiblast and describes an oval (Wetzel, 1929).

Free mesoderm still does not extend very far laterally. The anterior or preaxial mesoderm has grown somewhat farther forward (Fig. 55-C<sub>1</sub>).

### The Definitive Streak

The primitive streak of the chick reaches its full development at some time between the sixteenth and the twentieth hour of incubation, accord-



ing to Wetzel (1929), although Duval (1884a) recorded a somewhat earlier attainment of this stage. Similarly, Rabl (1923) and Chen (1932) differed as to the time the definitive streak is found in the duck's (*Anas platyrhynchos*) blastoderm, the former having observed it at the thirtieth hour, the latter at various times from the eighteenth to the twenty-fifth.

Incubation after the stage of the elongated streak accentuates the node and the groove. Where the groove takes its origin behind the node, there is now a deep depression, the primitive pit (Fig. 54-E); in the duck, *Anas platyrhynchos* (Fig. 51-C), however, the pit is often absent at this stage (Chen, 1932). Flanking the groove on either side are the primitive folds, which are high anteriorly and increasingly lower posteriorly as the groove flattens and widens. The embryonic shield is still present around the anterior half of the streak, but it has become oval rather than circular in shape and is now recognizable as the medullary plate (Fig. 52-C).

The streak has continued to grow and, in the chick blastoderm, may be from 1.5 mm. to 2.3 or 2.5 mm. long at this stage (Wetzel, 1929; Hunt, 1932; Dalton, 1935). It usually is equivalent to about three fourths of the length of the area pellucida, this dimension having increased to 3.0 or 3.5 mm. (Wetzel, 1929). The caudal end of the streak lies within the area pellucida and may appear bifurcated, particularly in the chick (Fig. 54-E).

After the anterior end of the streak reaches the center of the area pellucida, intussusceptive growth in the posterior half of the streak probably accounts for all further elongation (Spratt, 1946). As the table shows (Spratt, 1946), the original streak and subsequent additions make up the total length of the definitive streak in proportions that are considerably different from those observed during the stage of the medium streak.

Region of Growth	Proportion of Total Length (per cent)
Anterior	19.79
Posterior	6.91
Intussusceptive	46.70
Original streak	26.60

Cross sections of the definitive streak (Fig. 55-D<sub>1</sub> to D<sub>4</sub>) show that the node is its thickest and densest part. Just behind the node, the streak is least dense; throughout its posterior half, it gradually becomes broader, flatter, and again dense. The hypoblast is a continuous layer throughout the area pellucida. The preaxial mesoblast, now 0.2 mm. long (Wetzel, 1929), has begun to grow laterally, and its lateral portions are continuous posteriorly with the lateral mesoblast. On either side, the latter consists of a loose aggregation of stellate cells migrating peripherally along the upper surface of the hypoblast (Chen, 1932). At levels of the blastoderm posterior

to the midpoint of the streak, mesoblast has spread throughout the area pellucida and has penetrated into the area opaca (Fig. 57-A). The mesoblast posterior to the primitive streak has been called metastomal mesoderm.

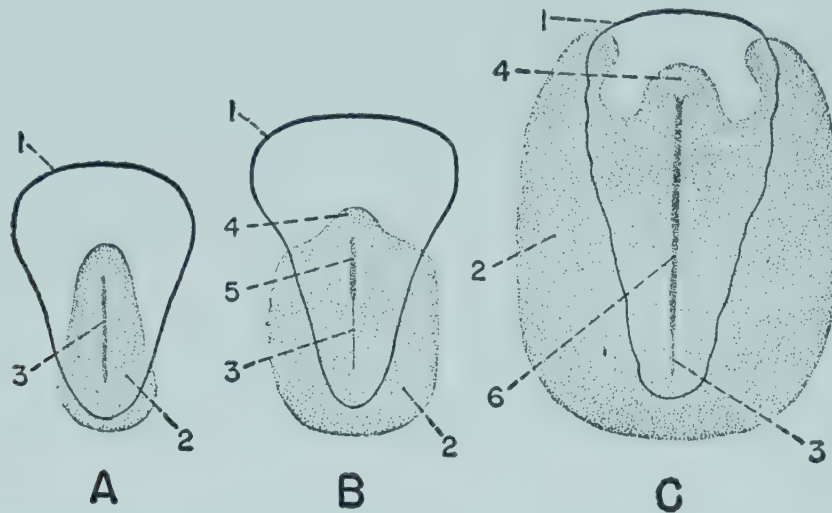


Fig. 57. Diagrams showing the growth of the mesoderm during the early development of the chick, *Gallus gallus*. (Redrawn after Adelmann, 1922.)

A, in a late primitive streak stage; B, in the head-process stage; C, after the formation of the notochord.

1, boundary of area pellucida; 2, mesoderm; 3, primitive streak; 4, preaxial mesoderm; 5, head-process; 6, notochord.

### The Origin of the Primitive Streak and the Mesoblast

Opinions regarding the origin of the primitive streak and the mesoblast have been as diverse as those offered to explain the formation of the hypoblast. It was not clear to many early investigators that the mesoblast was secondary to the streak; on the contrary, the reverse was frequently stated to be true.

In general, three different suggestions were made regarding the possible origin of the middle germ layer. The hypoblast was most widely accepted as its source (Remak, 1855, p. 6; Dursy, 1866, pp. 16–17; Goette, 1874; Disse, 1878), but some authors remarked that the epiblast also contributed elements to it (His, 1868, p. 72; Balfour and Deighton, 1882), and at least one considered the epiblast alone to be its forerunner (Reichert, 1840). Peremeschko (1868) and Oellacher (1872) believed that bodies migrated from the floor of the subgerminal cavity and through the germ wall to form mesoblast. Balfour (1873a) thought that “formative elements” fell from the blastoderm to the floor of the segmentation cavity, then migrated back between epiblast and hypoblast, where they joined with cells remaining in this position, to form mesoblast. Kölliker (1875, 1879, pp. 92–96) and Koller (1882), however, recognized the fact that the mesoblast grew centrifugally from the primitive streak.

The formation of the primitive streak has usually been attributed either to cellular proliferation *in situ* or to movements of cells or of large groups



of cells. A few other explanations have been offered; for example, the suggestion that the streak was the result of failure of the epiblast and the hypoblast to separate in the midline (*His*, 1868, *p.* 61), and the proposal that it developed through the fusion of mesoblast to epiblast in the same region (*Peremeschko*, 1868). Balfour (1873*a*), in his description of mesoblast formation stated that the conversion of "formative elements" occurred first in the center of the blastoderm, thus producing the primitive streak. The majority of nineteenth century workers, however, viewed the streak as a localized thickening of epiblast (*Kölliker*, 1875, 1879, *pp.* 92–96; *Gerlach*, 1881; *Balfour and Deighton*, 1882; *Koller*, 1882; *Mitrophanow*, 1899) or of both epiblast and hypoblast (*Remak*, 1855, *pp.* 7–8; *Dursy*, 1866; *Waldeyer*, 1869). At the turn of the century, the concept of primitive streak formation *in situ* was upheld mainly by *Assheton* (1896), *Kopsch* (1898, 1902), and *Peebles* (1898, 1904).

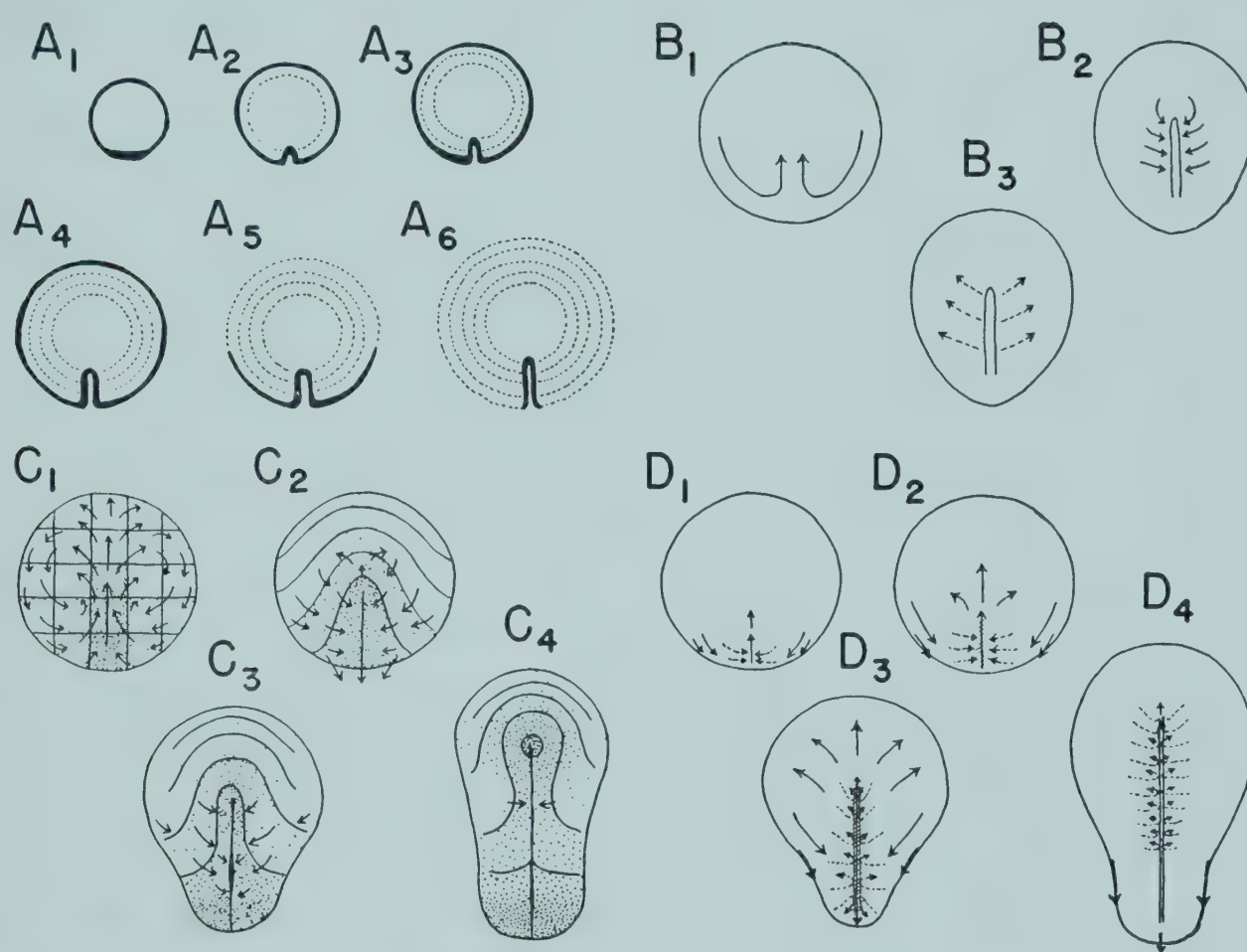
A few embryologists suggested that the primitive streak developed as the result of cell movements in the blastoderm. *Goette* (1874) and *Disse* (1878) believed that cells wandered centripetally into the area pellucida from the germ wall, to become first hypoblast and then mesoblast, the streak being an accumulation of these cells in the midline. Until well into the twentieth century, however, there existed only one important theory of primitive streak formation which involved cellular migrations; this was the famous "concrecence theory," according to which the streak develops when the margins of the blastoderm grow together at a certain point. *His* (1876*a*) first proposed the theory to explain the origin of the streak in animals lower than birds—teleost fishes, in particular. The next year (1877), he stated that he was unable to find evidence that the primitive streak of the chick was produced by any process other than the invagination of sagittal epiblastic folds. *Rauber* (1876*a*) and *Duval* (1884*a*) are chiefly responsible for the application of the concrecence theory to birds.

According to *Rauber* (1876*b*), the forerunner of the primitive streak is a crescent-shaped region located in the posterior part of the area pellucida and extending into the germ wall. The central part of this crescent, he thought, moved inward along the midline of the area pellucida, pulling the lateral portions of the crescent in its wake to form the primitive streak. A similar crescentic area, containing a transverse groove, was described by *Koller* (1879, 1882).

*Duval* (1884*a*) presented a somewhat more elaborate theory based on evidence he believed he had found. He stated that the entire rim of the blastoderm grows together in the midline to form the primitive streak. In his opinion, this concrecence occurs because the growth of the blastoderm is halted or considerably retarded at the midpoint of the posterior border. As the blastoderm expands elsewhere, it is inevitable that the portions of the border lying on either side of the stationary point should be folded to-



gether. As increasingly distant portions of the blastoderm make contact with each other, a line of fusion forms in the posterior midline of the enlarging blastoderm. In this way, the border of the blastoderm is transformed into an axial, linear structure, which lies within the blastoderm and grows by additions to its distal end. Duval's diagrams illustrating this process are reproduced in Fig. 58-A<sub>1</sub> to A<sub>6</sub>, inclusive. The account of hypoblast formation given by Patterson (1909*b*) tended to confirm Duval's theory, although Assheton (1896) failed to detect any convergence of the margins of blastoderms marked with sable hairs.



**Fig. 58.** Diagrams illustrating theories of primitive streak formation.

A<sub>1</sub>–A<sub>6</sub>, the concrescence theory of Duval (1884a); B<sub>1</sub>–B<sub>3</sub>, the concept of a “polonaise” movement of epiblast cells proposed by Gräper (1929); C<sub>1</sub>–C<sub>4</sub>, the theory of a “double vortex” of epiblast cells, conceived by Wetzel (1929); D<sub>1</sub>–D<sub>4</sub>, Wetzel's theory after modification by Pasteels (1937).

Many years later, the concrescence theory was re-examined. On the basis of experimental findings, it was first revised and then replaced, eventually, by a concept of primitive streak formation that is fundamentally different.

The first significant research on the problem was performed by Gräper (1929), who took stereophotographs of vitally stained blastoderms and observed a posteriorly directed movement of epiblast cells along the edges of the area pellucida, both right and left. At the midpoint of the posterior border, the two streams of moving cells apparently met, turned, and proceeded anteriorly along the midline (Fig. 58-B<sub>1</sub>), where they invaginated (Fig. 58-B<sub>2</sub> and B<sub>3</sub>). This evidence seemed to establish the fact of con-



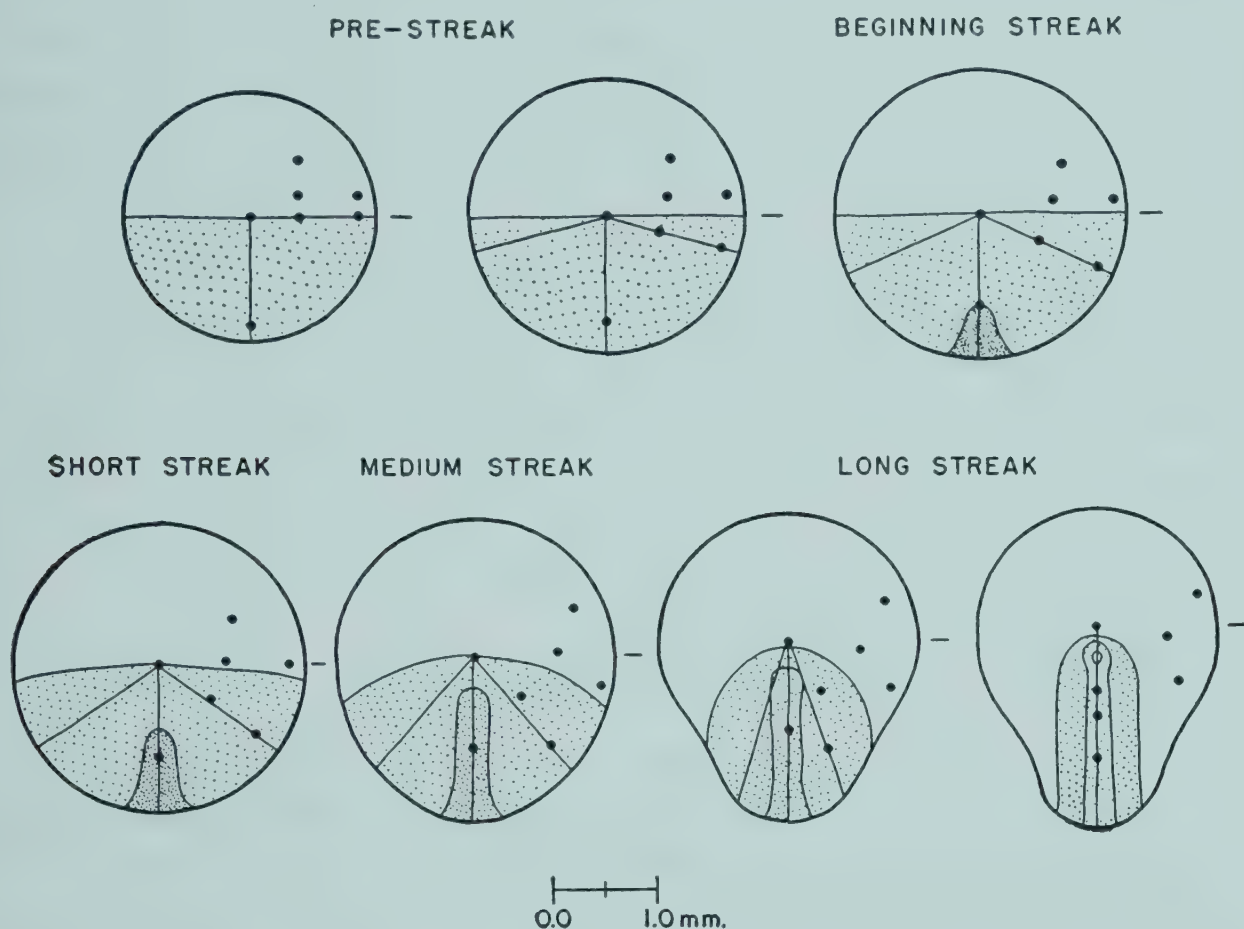
crecence, but, as Wetzel (1932) remarked, of the borders of the area pellucida rather than of the borders of the blastoderm.

Through the use of vital stains as isolated marks on the blastoderm, Wetzel (1929) discovered that the displacement of cells was even more complex than Gräper had indicated. According to Wetzel, there is a double vortex on either side of the midline, the vortex on the right moving in a clockwise direction, that on the left proceeding counterclockwise; in the midline, cells migrate cranialward (Fig. 58-C<sub>1</sub>). As the superficial cells invaginate through the streak (Fig. 58-C<sub>2</sub>), and as the streak elongates in a posterior direction (Fig. 58-C<sub>3</sub>), the cellular movements become restricted to the caudal half of the blastoderm. The last cells to invaginate enter the streak in its midportion (Fig. 58-C<sub>4</sub>).

Pasteels (1937), after modifying the technique of vital staining to eliminate some of its uncertainties, found evidence that there are two types of cellular movement in the area pellucida. One type, it appears, involves both epiblast and hypoblast simultaneously, and largely accounts for the pattern of cell displacements outlined by Wetzel (1929). The anterolateral movement in the anterior portion of each vortex is thus explained as a dislocation of both layers due to the forward thrust of the growing primitive streak. The other type of cell movement Pasteels termed truly morphogenetic; it consists of a streaming of cells of the epiblast alone toward the midline, where the cells invaginate to form the primitive streak. Whereas Wetzel attributed the initial appearance of the primitive streak to a proliferation *in situ* of material already located in the midline, Pasteels suggested that the streak is formed, from the very beginning, of cells originally situated elsewhere. The latter author believed that the first cells to migrate medially were those located in the posterior one fifth of the area pellucida. Figures 58-D<sub>1</sub> to D<sub>4</sub>, inclusive, indicate Pasteels' conception of cellular movements in the blastoderm from the inception of the streak to its definitive stage.

As Spratt (1946) has shown, the cellular movements leading to the formation of the primitive streak can be studied with even more precision by the use of powdered blood carbon marks. This method appears to be superior to vital staining, inasmuch as dyes tend to diffuse from one embryonic layer to another and are taken up selectively by different cells of the same layer. The results of carbon-marking experiments indicate that there is only a slight anterior movement in the streak proper, this movement most probably being an anterior migration of hypoblast and possibly of invaginated elements, not of epiblast. Although there is also an anterolateral movement of material away from the midline of the anterior portion of the area pellucida, as Wetzel indicated, this displacement appears to be of the epiblast, and not a "simultaneous movement" of both layers, as Pasteels termed it. On each side of the blastoderm, the movements seem to follow the course of radii sweeping from the anterior midline to the

posterior midline. The speed of the moving cells on these radii is a direct function of the distance of the cells from the center of the area pellucida, for the cells located at the periphery move at the most rapid rate, those close to the center at the slowest. Figure 59 indicates the manner in which epiblastic material is brought to the primitive streak, as visualized by Spratt (1946). It may be seen that cell migration occurs throughout about two thirds of the area pellucida, although only the posterior half of the epiblast is invaginated by the stage of the definitive streak. Wetzel and Pasteels believed that no more than a quarter of the surface of the area pellucida enters the primitive streak.



**Fig. 59.** The movements of epiblastic material which form the primitive streak, as shown by diagrams indicating successive stages in the displacement of seven carbon marks on the surface of the epiblast. (Redrawn after Spratt, 1946.)

Solid black circles indicate carbon marks; the dash, a reference point; light stippling, the area to be invaginated; close stippling, the primitive streak. All in scale.

If it is accepted that 50 per cent of the material occupying the surface of the pellucid area turns downward through the streak, the question arises as to why the area pellucida does not contract in size during the early stages of streak formation. In answer, there is evidence (Derrick, 1937; Spratt, 1946) to show that mitotic activity and cellular proliferation are sufficiently vigorous throughout the epiblast—and particularly in the anterior and peripheral portions of the area pellucida—to compensate for the invaginated areas. According to Chen (1932) and Spratt (1946), the streak is thus the product of both the proliferation and the migration of cells. On the other hand, Pasteels (1937, 1943) found that mitotic activity does not differ



significantly in the epiblast and the primitive streak of the chick and the duck, *Anas platyrhynchos*; he therefore concluded that cellular movements alone cause the formation of the streak.

Both Pasteels (1937) and Twiesselmann (1938) remarked that the cellular movements do not begin until the appearance of the streak is imminent. Spratt (1946) placed their inception at about the sixth hour of incubation.

### The Regression of the Primitive Streak

Soon after reaching its definitive length, the primitive streak starts to grow shorter, both absolutely and in relation to the size of the elongating area pellucida. Simultaneously, it retreats posteriorly. In the chick, the streak recedes and diminishes until some time between the thirty-sixth (*Balfour, 1873b*) and the forty-ninth (*Dursy, 1866*) hour of incubation, while the primordial embryonic body forms in its wake. At about the 20-somite stage, the remaining material of the streak is transformed into a terminal swelling known as the end bud or (later) as the tail bud, which has also been called the "trunk-tail bud" (*Holmdahl, 1951*). It now seems that the streak may remain active, through the end bud, until a much later period than previously believed. It is therefore necessary to indicate the course of events that takes place well after organogenesis begins, in order to complete the history of the primitive streak.

### The Early Head-Process Stage

The regression of the streak begins soon after the appearance of the so-called "head-process," which is a thick, short, somewhat pointed aggregation of cells projecting forward from the primitive node (Fig. 51-D; Fig. 54-F). The head-process has been said to form in the chick at various times from the sixteenth (*Balfour, 1873b; Adelmann, 1922*) or nineteenth (*Duval, 1884a*) hour of incubation to "the end of the first day" (*Wetzel, 1929*). In the duck (*Anas platyrhynchos*), it becomes visible at some time between the twenty-fourth (*Chen, 1932*) and the thirtieth hour (*Rabl, 1923*). This structure is misnamed, since it is the primordium of the notochord (chorda) and will take part in the development of the vertebral column, not of the head.

The head-process, which is composed of several layers of mesoblast cells, appears to originate from the deeper portion of the node (Fig. 52-D). It is flanked on either side by mesoblast (Fig. 57-B), which was termed the "gastral mesoderm" by Rabl (1889a). At its anterior extremity, the head-process is continuous with the prechordal plate, a mass of loosely arranged cells that has developed from the preaxial mesoderm (*Adelmann, 1922*) and is closely bound to the hypoblast. Spratt (1947b) noted that there is fusion of all three germ layers at the anterior end of the early head-process



and that this fusion persists until the 10-somite stage. He also obtained good evidence that the head-process is molded *in situ* from the sheet of previously invaginated mesoblast cells. This finding is in agreement with the observations of Kuhlenbeck (1930).

Above the prechordal plate and the head-process, the embryonic shield (neural or medullary plate) has become thickened to a pseudostratified epithelium with nuclei in two or three layers (cf. Fig. 52-D). In the node region, the hypoblast is still fused to the overlying cells (Schauinsland, 1899; Hertwig, 1906, p. 870).

### The Late Head-Process Stage

As the compact chorda primordium elongates, cellular activity in the medullary plate is intensified. By the time the head-process has attained a length of 0.6 mm. (Rawles, 1936), the anterior edge of the plate thickens

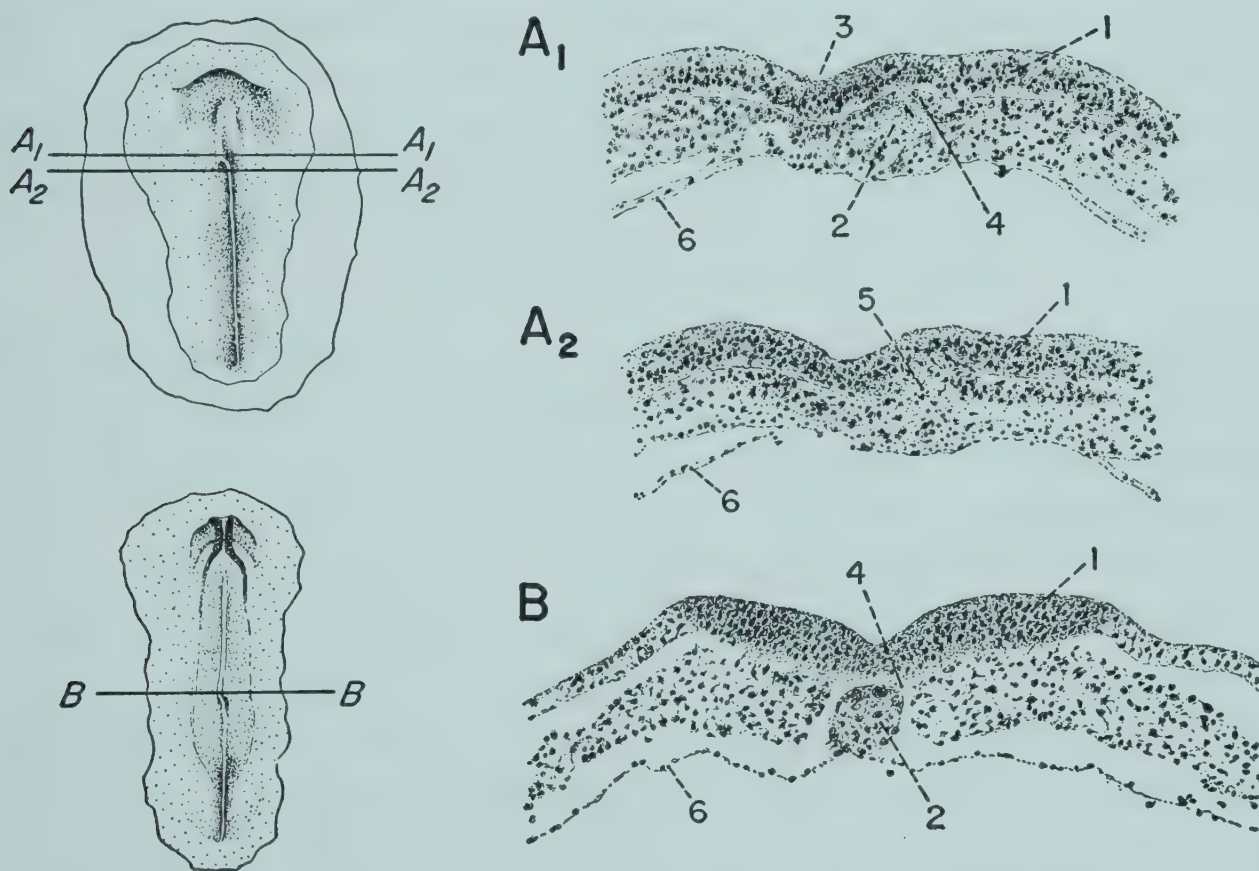


Fig. 60. The differentiation of the notochord from the head-process, and the relationship of both structures to the medullary plate and groove and to the primitive node, as seen in the cross sections. (Redrawn with modifications from Wetzel, 1929.)

A<sub>1</sub>, A<sub>2</sub>, at the early head fold stage; B, at the 3-somite stage. All  $\times 110$ . The levels of the sections are indicated on the surface views at the left.

1, neural plate; 2, chorda anlage or notochord; 3, groove lateral to the primordial notochord; 4, fissure between neural plate and mesoderm or notochord; 5, region where ectoderm and mesoderm are continuous; 6, endoderm.

and rises up from the surface of the blastoderm as a curved ridge whose convexity is directed anteriorly. Together, the head-process and the medullary plate now appear as an anchor-shaped complex when viewed from above (Fig. 51-E). The medullary plate narrows posteriorly and terminates



about halfway down the streak (Fig. 54-G). It is bisected longitudinally by the broad, shallow medullary (neural) groove, on each side of which is a suggestion of a medullary (neural) fold. Usually the right fold is somewhat more pronounced than the left, and the medullary groove lies slightly to the left of the midline. The medullary groove becomes more shallow as it approaches the node (Fig. 60-A<sub>1</sub>) and is continuous with the primitive pit. The latter has deepened markedly and now divides the node diagonally into a large segment on the right and a smaller one on the left (Fig. 60-A<sub>2</sub>). Wetzel (1929) noted that a line of separation between epiblast and mesoblast is beginning to appear throughout the medullary plate (cf. Fig. 60-A<sub>1</sub>). The head-process adheres ventrally to the hypoblast and dorsally to the ventral surface of the epiblast that forms the floor of the medullary groove. The chorda primordium is continuous at its posterior extremity with the node and is gradually differentiating as a rod-shaped entity. The mesoblast flanking the head-process laterally has become loosened from the hypoblast (Adelmann, 1922), but, at levels between the middle and the posterior end of the streak, mesoblast and hypoblast are still adherent (Wetzel, 1929). Mesoblast now reaches well into the area opaca. The various regions of the primitive streak proper appear essentially unchanged.

### *The Head Fold Stage*

The medullary plate bulges forward to a continually increasing extent, until it forms an overhanging projection. The indentation beneath this projection is known as the head fold. Figures 51-F and 54-H give surface views of blastoderms at this stage of development.

The hypoblast is carried forward with the epiblast of the medullary plate, but it is so intimately fused with the mesoblast of the prechordal plate as to be indistinguishable from the latter (Adelmann, 1922). The hypoblast anterior to the prechordal plate is folded under and is incorporated into the roof of the head fold. The roof and floor of the head fold, as well as the area pellucida immediately anterior to it, consist only of epiblast and hypoblast. The mesoderm-free area of the area pellucida is known rather misleadingly as the proamnion. More posteriorly, the mesoblast has increased in extent (cf. Fig. 57-B); it has reached the margins of the area pellucida anterior to the level of the primitive pit and is progressing into the area opaca at all more posterior points. The mesoblast within the area opaca has become greatly thickened in certain spots; these areas are "blood islands" (see Chapter 13).

According to Wetzel (1929), the invagination of cells into the greater part of the streak ceases after the formation of the head-process but continues in the midportion of the streak up to the stage of the head fold. There is evidence, however, that cells are still invaginating at a much later period (Spratt, 1947b; Spratt and Condon, 1947).



*Late Stages of Regression*

As the streak continues to regress, the notochord differentiates from its primordial condition and is clearly visible as an elongating, rod-shaped median structure (Fig. 60-B) anterior to the node and proceeding out of the latter. At either side of the notochord, the paired somites or proto-vertebrae become molded out of the mesoblast closest to the midline. The first pair of somites appears in the chick blastoderm shortly before or after the end of the first day of incubation. Additional pairs of somites differentiate in succession, each pair close behind the last-formed pair.

Anteriorly, mesoblast begins to grow forward on either side of the proamnion (Fig. 57-C) and eventually (at some time between the 9- and the 14-somite stage) surrounds it completely. Beginning at about the 3-somite stage, the mesoblast gradually splits into an upper and a lower layer, the somatic and the splanchnic mesoderm, respectively. The somatic mesoderm and the ectoderm are known as the somatopleure, and the splanchnic mesoderm and the endoderm as the splanchnopleure. The space between somatopleure and splanchnopleure is the coelom, or body cavity. At a level immediately anterior to the anterior intestinal portal, the coelom on each side is much deeper than elsewhere, forming bilateral dilations known as the amniocardiac vesicles. Soon the mesoderm containing the amniocardiac vesicles swings toward the midline, growing between the ectoderm of the subcephalic pocket and the endoderm of the fore-gut. At the stage of 4 or 5 somites, the medial walls of the amniocardiac vesicles come into contact with each other and establish the primordium of the heart from their splanchnic mesodermal components (see Chapter 13). At about the 9- to 12-somite stage, the amniocardiac vesicles become continuous from side to side, forming the primitive pericardial cavity. At more posterior levels, the coelom still consists of bilateral halves. Its enclosing mesodermal layers (somatic and splanchnic) join medially to form a narrow zone of undivided mesoderm, the intermediate mesoderm or nephrotome, which lies immediately lateral to the somite mesoderm.

The neural folds along the lateral borders of the neural plate grow higher and gradually approach each other, beginning anteriorly. By fusion in the midline, above the notochord, they form the closed, hollow neural tube. The more posterior portion of the notochord is flanked by the backward-growing neural folds. Anteriorly, the neural tube enlarges to form the three primary vesicles of the brain (see Chapter 4).

Meantime, the cephalic extremity of the body has continued to grow forward, deepening the head fold and subcephalic pocket, and becomes recognizable as the head. The endoderm that has been carried into the head is the early fore-gut, or anterior portion of the embryonic gut. The fore-gut has the form of a pocket open to the yolk posteriorly; its posterior limit is

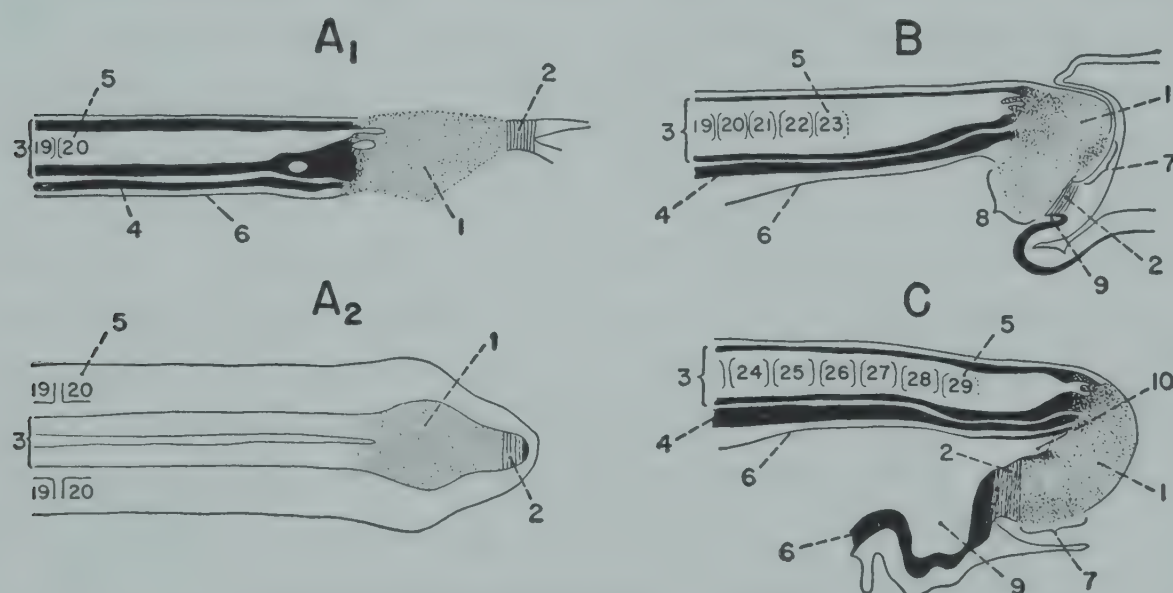


termed the anterior intestinal portal. The fore-gut elongates from anterior to posterior by the apposition and fusion in the midline of two folds of splanchnopleure, one on either side.

The embryonic body is gradually delimited from the extraembryonic part of the blastoderm by the appearance of a longitudinal groove in the somatopleure on each side, some distance lateral to the somites. These grooves, which form from anterior to posterior, are caudal continuations of the head fold, and more or less parallel the splanchnopleuric folds that produce the fore-gut. The folding of the flat blastoderm reveals that formerly lateral areas are to be incorporated into the ventral part of the body.

### *End Bud and Tail Bud Stages*

By the time twenty somites have appeared, the remnant of the primitive streak enlarges to form the end bud, which is the forerunner of the tail bud. There is no absolute agreement among embryologists as to exactly which



**Fig. 61.** The disappearance of the primitive streak and the formation of the tail bud from the end bud of the chick embryo, represented diagrammatically. (Redrawn with modifications after Holmdahl, 1925a.)

A<sub>1</sub>, sagittal section, end bud stage; A<sub>2</sub>, surface view, end bud stage; B, sagittal section of tail bud with beginning ventral flexure; C, sagittal section of tail bud after formation of tail fold. All  $\times 33$ .

1, end bud or tail bud; 2, primordium of cloacal membrane; 3, neural tube; 4, notochord; 5, last somite (indicated also by its number); 6, endoderm; 7, vestigial primitive streak; 8, ventral remnant of primitive streak; 9, primordium of allantois; 10, tail gut.

portion of the streak enters into the end bud and tail bud. Kopsch (1934b) believed that material located in the midportion and third quarter of the definitive streak was destined to form the tail bud. To Wetzel (1932) and Pasteels (1937), on the other hand, the end bud represented the entire streak, greatly shortened. Spratt (1947b) visualized the end bud as being composed of the node, together with condensed or compressed material originally situated at anterior levels of the streak (an explanation of the

fact that the end bud is larger than it would be if composed of the node alone).

Holmdahl (1925*a*), in describing the end bud stage in the chick, noted that the end bud, when seen from the surface, gives the false appearance of being a swelling of the neural tube. A sagittal section, however, shows that not only the neural tube but also the ectoderm, endoderm, and chorda proceed directly out of the end bud (Fig. 61-A<sub>1</sub> and A<sub>2</sub>). It can be shown that ectoderm, endoderm, and mesoderm also go out laterally from the end bud.

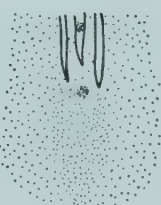


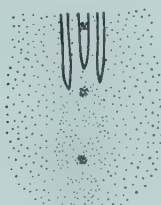


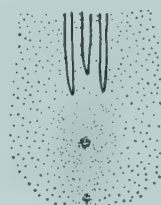


	TYPE OF MARKING		RESULTS
	DORSAL VIEW	SAGITTAL VIEW	
A			
B			
C			

Fig. 62. Diagrams showing the caudal region of 2-day chick embryos marked with carbon particles (first two columns) and the appearance of the marks 2 days later (third column, drawn to a smaller scale). (Redrawn with modifications from Gaertner, 1949.)

A, a mark at the anterior border of the end bud elongates, whereas a mark on the body of the embryo is unchanged; B, a mark made on the posterior portion of the end bud also increases in size; C, a mark at the extreme caudal tip of the end bud remains unaltered.

During the period when the twenty-third to twenty-ninth somites develop, the end bud gradually becomes the tail bud. According to Holmdahl (1925*a*), several phenomena characterize this change. Primarily, there is an enlargement of the end bud, followed by a ventral flexure of its posterior portion (Fig. 61-B). This flexure forms the tail fold and brings what Holmdahl identified as the much reduced primitive streak into such a position that it is invisible from above (Fig. 61-C). The ectoderm in the midline of the streak separates from the cell mass of the tail bud, with which it was formerly fused, and the endoderm of the streak forms the primordium of



the tail gut. The streak thus disappears. In addition, germ layer formation ceases with the establishment of the tail bud.

Studies of the end bud by means of carbon marks have yielded interesting results (Gaertner, 1949). A mark placed in the differentiated region anterior to the end bud remains unchanged in size and shape after further incubation, whereas one at the borderline between the end bud and the differentiated region becomes greatly elongated (Fig. 62-A). A mark located more posteriorly in the end bud also grows longer (Fig. 62-B), but one placed at the extreme caudal end of the end bud does not increase in size (Fig. 62-C). These findings appear to indicate that the end bud, at its anterior border, is continually differentiating into the structures of the embryonic body (the formation of the tail fold resulting from the fact that the caudal end of the end bud represents a fixed point). Inasmuch as mitotic counts reveal that the end bud is not a center of cellular proliferation (Pasteels, 1943; Gaertner, 1949), it appears that the substance of the end bud merely rearranges itself. As a result, the end bud, like the primitive streak, moves to the rear as it decreases in size (Gaertner, 1949).

### *The Mechanism of Regression*

There has been much discussion regarding the process underlying the shortening and regression of the streak. Marking the epiblast with carbon powder has assisted in clarifying the matter. Spratt (1947b) was thus able to show fairly definitely that the streak decreases in length principally through the shortening of its posterior half. Balfour (1885, pp. 165-166) also drew this conclusion from a study of serial sections. As Fig. 63 shows,

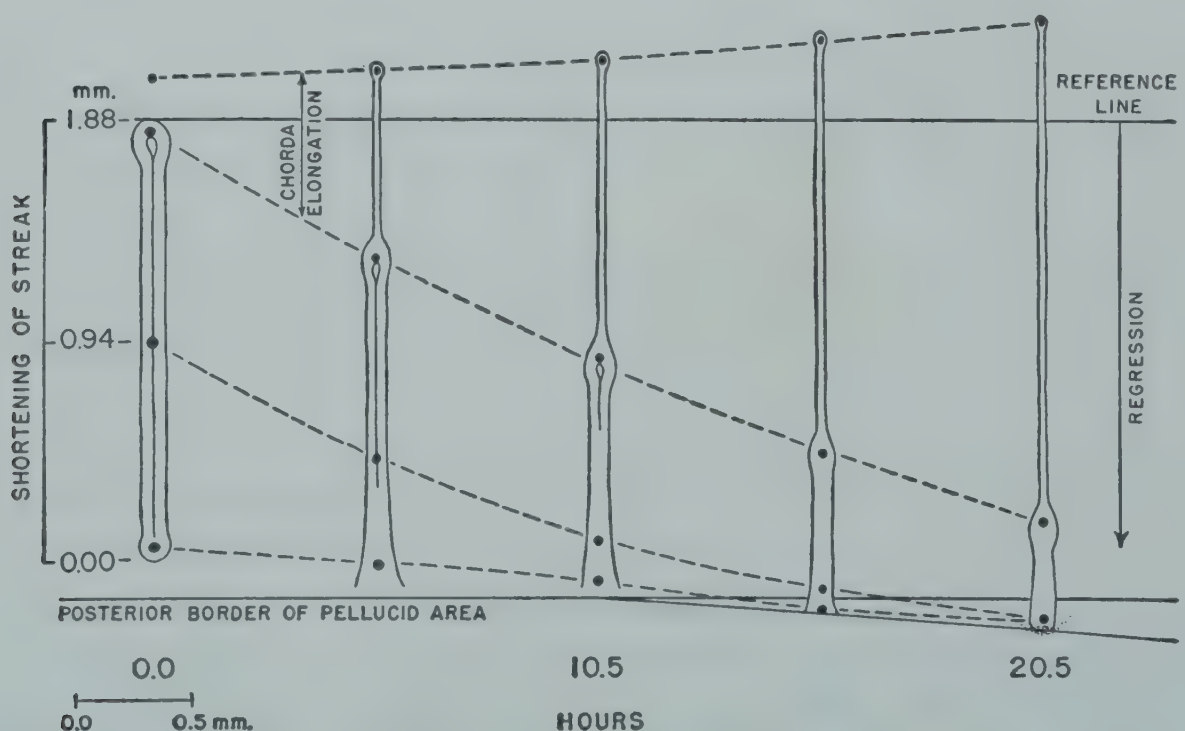


Fig. 63. Diagram, in scale, illustrating regression and shortening of the primitive streak and elongation of the notochord in the chick embryo. The slopes of the broken lines indicate the rates of movement of the marked cell groups (black circles). (Redrawn after Spratt, 1947b.)

the anterior half of the streak does not decrease in length until the posterior half has almost disappeared. This figure indicates the changing position of carbon marks at the anterior and posterior ends and at the midlevel of the regressing streak, as revealed by experiments with explanted blastoderms; the elongation of the chorda is also depicted.

Apparently the posterior end of the streak grows shorter because its dorsal portion spreads out laterally and posteriorly to form ectoderm, most of which seems to be extraembryonic. Mesoderm also spreads out in the extraembryonic region lateral and posterior to the streak. Ectoderm probably continues to invaginate (Fig. 64) during the entire period of regression (Spratt, 1947b; Spratt and Condon, 1947), rather than ceasing to do so during the head-process stage, as Wetzel (1929) thought.

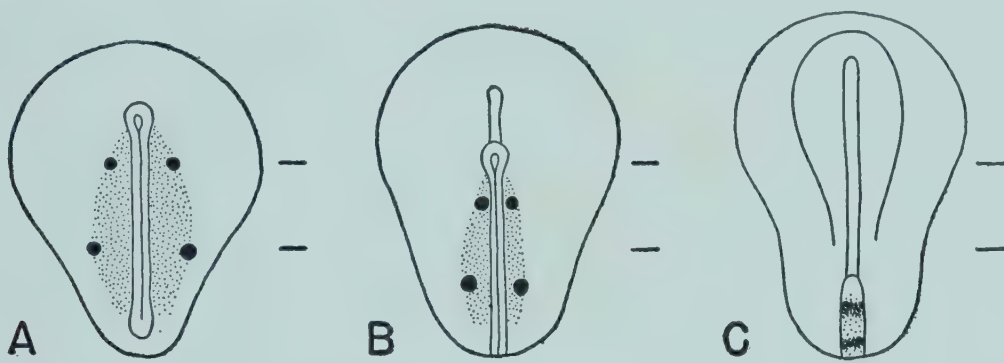


Fig. 64. Diagrams showing the invagination of epiblast during the regression of the primitive streak in the chick embryo. (Redrawn after Spratt, 1947b.)

A, carbon marks (black circles) on surface of blastoderm in the definitive primitive streak stage (area destined to invaginate is stippled); B, posteromedial movement of carbon marks (shown by position relative to two reference marks in the area opaca) and invagination of epiblast after regression of the streak starts; C, inclusion of the marks and of surface epiblast within the streak in an advanced stage of regression.

While the posterior half of the streak grows shorter, it is also receding; but the anterior half regresses at a more rapid rate (Fig. 63), as Gräper (1929) noted. The average rate of regression of the node and the anterior half of the streak is 0.08 mm. per hour for 20 hours, as against 0.033 mm. per hour for the posterior streak during the same period (Spratt, 1947b).

Spratt and Condon (1947) found that carbon-marked superficial cells on the node and just behind it undergo a true regression, slipping over the somite material. As the node regresses, the area pellucida anterior to it increases in length. This anterior portion of the area pellucida, keeping pace with the rapid elongation of the notochord, grows at a much faster rate than the area pellucida as a whole. The growth rate of the anterior area pellucida parallels the rate of regression of the node (Spratt, 1947b). It appears, therefore, that the cranial end of the streak is pushed to the rear by the growth of the embryonic body ahead of it, increasingly anterior levels of the streak moving back into the future tail region as the posterior end of



the streak is transformed into embryonic and extraembryonic ectoderm and mesoderm. Spratt (1947*b*) therefore suggested that regression of the streak is a relatively passive movement, as compared with the elongation of the notochord, which is an autonomous movement. Furthermore, the regression of the streak is not an illusion, as suggested by Jacobson (1938*b*), but an actuality, as indicated by Balfour (1873*b*).

Not only the streak, but the regions lateral to it undergo regression; that is, the ectoderm, mesoderm, and endoderm move backward in the portions of the area pellucida that flank the streak. All three layers seem to recede at the same rate. This rate, however, varies directly with the distance from the streak. Cells close to the streak regress almost as rapidly as the streak itself, while those at the outer limits of the area pellucida scarcely move or remain entirely stationary (Spratt, 1947*b*).

### Gastrulation and the Blastoporal Value of the Primitive Streak

The process known as gastrulation has been interpreted in various ways by embryologists. As Peter (1938*a*) remarked, there have been three general definitions of gastrulation. First, it has been considered merely as the division of the primitive embryo (blastula) into ectoderm and the endoderm that is to line the gut; second, it has been looked upon as the establishment of the archenteron (primitive gut cavity) or a comparable structure; and third, it has been regarded as the movement to the interior of those cells which will give rise to the gut endoderm, the mesoderm, and the chorda (notochord).

The last view, which is coming to be accepted to the exclusion of others, permits gastrulation to be conceived as a primarily kinetic phenomenon involving a transferral of cells from the surface to the inside. As Pasteels (1940, 1945) pointed out, comparative embryology reveals the difficulties attendant upon a definition in morphological terms, for the forms produced by gastrulation are extremely varied. The one constant characteristic of the process is the movement of surface cells to the interior.

The question of exactly what ontogenetic process constitutes gastrulation in birds has been a cause of controversy for many years and is not yet entirely settled. On the basis of morphological evidence, the avian blastoderm at different stages has been homologized with the gastrulae of various lower forms. In general, however, there are only two periods in avian development that have been considered as gastrulation.

The first of these periods is the time during which the primary endoderm is formed. In relatively simple types of gastrulation, the infolding or invagination of external cells almost obliterates the segmentation cavity while creating a new cavity, the archenteron (gastrocoele or coelenteron). The invaginated cells constitute the endoderm—the lining of the archenteron, of which the blastopore is the mouth. Around the blastopore there is great



cellular activity; and subsequent events reveal that the three primary germ layers are fused at the anterior or dorsal blastoporal lip.

It is possible to interpret the bilaminar avian embryo as a flattened gastrula, in which the fissure between ectoderm and endoderm corresponds to the segmentation cavity, the subgerminal cavity being transformed *in situ* into the archenteron. If the endoderm arose by invagination around the entire periphery of the blastoderm, as Goette (1874) and Disse (1878) suggested, then the marginal region becomes comparable to an extended blastopore. Duval (1884a) and Patterson (1909b), on the other hand, limited the blastopore to the posterior border, for, as we have seen, they believed the invagination of endoderm to be confined to this location. In their opinion, the posterior margin of the blastoderm corresponds to the dorsal lip of the blastopore in the amphibian egg; the yolk, by homology, is the ventral lip; and the narrow intervening space is the blastopore, continuous with the archenteron. Patterson, observing two horns of the zone of junction approaching each other posterior to the site of invagination in the pigeon's (*Columba livia*) blastoderm, interpreted their fusion about 4 hours before egg-laying as closure of the blastopore.

There are obvious objections to the view held by Duval and Patterson. As Assheton (1912) remarked, if the yolk is the ventral lip of the blastopore, then the avian blastoderm is unique among vertebrate gastrulae. There is doubt, too, as to the actual existence of an archenteric cavity separating the yolk from the posterior rim of the blastoderm. Furthermore, many who accept delamination as the origin of the primary endoderm in birds do not consider the formation of this germ layer as gastrulation, for the fission of the blastoderm into two layers is not a cellular migration of the type that is usually required to produce a gastrula. To Pasteels (1940, 1945), for example, the bilaminar avian embryo is merely a blastula, the space between epiblast and hypoblast representing the blastocoele—an intercellular cleft produced by the separation of blastomeres. Pasteels and many others have denied that, in the words of Kionka (1894), any events in avian development prior to the formation of the primitive streak can be conceived as similar to gastrulation.

The stage of the primitive streak, therefore, is the second embryonic period that can be taken as gastrulation in birds. Cells invaginate through the primitive streak from the surface to the interior and probably continue to do so as long as the streak is in existence. In the kinetic sense, therefore, the primitive streak has a blastoporal value. It is not the strict counterpart of the blastopore of other animals because of the spatial and temporal rearrangements that distinguish avian development.

In the past, many embryologists recognized the similarities between the avian primitive streak and the blastopore of other forms. Regardless of whether they believed that the streak originated by proliferation or by



concrecence, they attempt to interpret it in the light of morphological evidence. Thus Balfour and Deighton (1882), representing one viewpoint, noted that the three primitive germ layers are fused at the anterior end of the streak, as they are at the dorsal lip of the blastopore. Representing the other viewpoint, Rauber (1876a) spoke of the primitive folds as continuations of the blastoporal lips, and Patterson (1909b) identified the streak as the fused halves of the dorsal lip.

Gräper (1929), the first to provide evidence of the morphogenetic movements producing the primitive streak, proposed a theory of gastrulation that includes many features of earlier views. He envisaged gastrulation in birds as biphasic, the first phase consisting of primary endoderm formation and the second of mesoderm (primitive streak) formation. Therefore, he was able to integrate his findings on birds into a system of gastrulation applicable to all vertebrates and comprising the following steps: (1) the placing of the hypoblast in the deepest position; (2) the movement of the chorda and mesoblast into a middle position; (3) the ranging of the chorda and mesoblast into craniocaudal order; and (4) the closure of the blastopore. The invagination of the hypoblast along the posterolateral border of the blastoderm (a process that he did not question) corresponded to the first point. The second and third stipulations were satisfied by the morphogenetic movements, which reversed the original order of mesoderm and chorda (cf. Fig. 58-B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>), and by the invagination of mesoblast through the streak to a position between epiblast and hypoblast. Since he regarded the streak as an open blastopore, the regression of Hensen's node fulfilled the fourth condition; to him, the laying down of the epiblastic floor of the neural tube was the closure of the blastopore.

A considerably different stand was taken by Peter (1938a), who saw gastrulation as the process giving rise not to gut endoderm and chordomesoderm, as Gräper (1929) conceived it, but to the primitive gut (archenteron) or a comparable structure. He found it impossible to include endoderm formation in avian gastrulation because his investigations furnished evidence that this germ layer does not arise by invagination and is an entirely new formation. He stated that any cell masses that penetrate to the interior can be homologized, whether or not they furnish intestinal epithelium. In his estimation, therefore, the solid head-process of birds is the homologue of the archenteron in amphioxus and *Amphibia*. This is much the same view as that of Schauinsland (1903a), who claimed to have seen a small canal leading from the primitive pit into the chorda of the albatross (*Diomedea immutabilis*), the gannet (*Sula piscatrix*), the frigatebird (*Fregata aquila*), the red-tailed tropic bird (*Phaëthon rubricauda*), and the shearwater (*Puffinus pacificus*). Schauinsland considered this canal to be a rudimentary archenteron and stated that the proliferation and invagination of the primitive streak (including the node) could be considered



as gastrulation leading only to mesoblast formation. Jacobson (1938*b*), however, attributed the “canalis chordalis” to mechanical causes and noted that it is not a formative structure.

Wetzel (1929, 1932) deviated considerably from other viewpoints. In his opinion, the primitive streak is a blastopore only in a limited sense and during its early existence, when free mesoderm is being formed; even then, it is only comparable to a blastopore, because it is not open. One of Wetzel's primary points is the “indifferent” character of the primitive streak, especially in its later stages. Therefore, it may be said that he, like Gräper, thought of gastrulation as biphasic; but Wetzel's first phase is “blastoporal” and his second, “indifferent.”

By contrast, Pasteels (1936, 1937, 1940) saw the primitive streak as in truth a blastopore, because he defined the blastopore as merely the point of

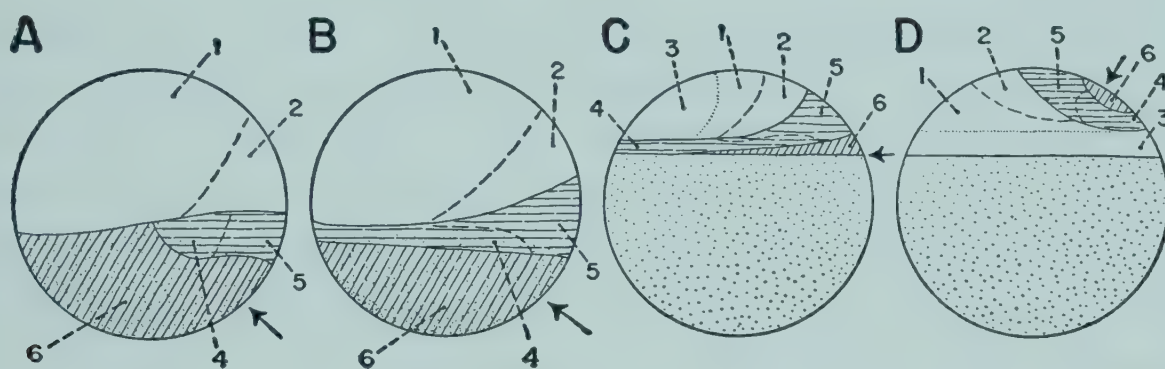


Fig. 65. Diagrams showing the disposition of presumptive areas in the blastulae of chordates. Each diagram represents the surface of half the blastula, seen in lateral view. (Redrawn after Pasteels, 1937.)

A, cyclostomes; B, anurans; C, teleosts; D, sauropsids. Presumptive chorda and mesoderm are both represented by horizontal lines, endoderm by oblique lines, and yolk by stippling. The arrow indicates the position of the blastopore.

1, ectoderm; 2, neural material; 3, extraembryonic ectoderm; 4, mesoderm; 5, chorda; 6, endoderm.

entrance to cells that invaginate. He regarded gastrulation as the migration and incorporation into the embryo of surface areas of the blastula which are destined to differentiate, each in a certain direction. In analyzing gastrulation from the comparative standpoint, he found only three constant characteristics of the process in all chordates: (1) the relationships between these areas (presumptive areas) on the surface of the blastula; (2) the morphogenetic movements; and (3) the result—the primitive embryonic body.

Figure 65 shows the approximate disposition of the presumptive areas in the blastulae of cyclostomes, anurans, teleosts, and sauropsids. (The sauropsidan blastula pictured is that of a reptile but will suffice for birds, also.) In every instance, the mesoderm is divided into two bilaterally symmetrical parts separated by the chorda; the neural material lies above the mesodermal areas. In all the anamniotes (that is, in all but the sauropsidan



blastula), the prospective endoderm is found in a lower position than the future mesoderm. In the cyclostomes (Fig. 65-A) and anurans (Fig. 65-B), the blastopore is within the endodermal area. In the microblastic teleosts (Fig. 65-C), it lies at the edge of the blastoderm, at the junction of endoderm and yolk, which are distinct from each other. Coming now to the sauropsids, one is immediately struck by the changed position of both the endodermal area and the blastopore. The endoderm is no longer in contact with the yolk mass and the blastopore is not marginal but central. The zone of extraembryonic ectoderm has displaced the mesodermal and endodermal areas to a medial position in the blastoderm and lies between them and the yolk. It can be seen, however, that the sauropsidan blastula is distinguished from the blastulae of other forms by differences in the relationships between the yolk and the presumptive areas, rather than by differences in the relationships of the presumptive areas to each other.

Insofar as the morphogenetic movements are concerned, Pasteels noted that they are the same in all chordates; that is, they consist of the cellular displacements first classified by Vogt (1925, 1929) for amphibians: invagination, extension, dorsal convergence, epiboly, and ventral divergence. The general applicability of these principles has forced the concept of concrescence into oblivion. The cellular displacements, however, do not always occur in the same order. Variations in their chronology, together with the slightly different spatial relationships between the presumptive areas, account for all the morphological dissimilarities in the results of gastrulation. There are no radical differences in gastrulation among anamniotes; but the amniotes, beginning with reptiles, are immediately set off from the lower forms by the altered sequence of events. The principal changes found in birds are as follows: (1) the primary endoderm is formed precociously, before the invagination of the chordomesoderm; (2) the order of invagination of chordomesoderm is reversed, ventral material preceding dorsal; (3) the movement of extension (starting with the regression of the node) is delayed until invagination is largely completed, instead of being synchronized with it. The morphogenetic movements continue in the tail bud, which is not a "bud" but the remnant of the gastrula (Pasteels, 1943).

According to Pasteels, a few generalized conclusions may be drawn regarding gastrulation. The archenteron is not a constant feature of the gastrula, and, when present, it is only a mosaic of chordomesodermal and endodermal elements; it does not exist at all in birds. The germ layers are not morphological entities but represent mere temporary maneuvers undertaken by cells on the march—a definition, incidentally, rejected by Holmdahl (1939a), who gave the germ layers the rank of independent primitive organs. The blastopore is variable in form but unchanging in its function of providing entrance to the interior; in birds, it is elongated and closed. It



follows that the closure of the blastopore does not universally occur and is not necessary; it cannot take place in birds because the blastopore is not open in the first place.

**The Presumptive Areas of the Avian Blastoderm.** The various concepts of gastrulation in birds have their origin in different experimental findings on both the original disposition of presumptive areas and the subsequent changes in the relationships between these areas. Through the use of vital stains or carbon particles to mark cell masses, the arrangement of future medullary plate, chorda, and mesoderm (prechordal, somitic, and lateral plate) in the pre-streak blastoderm has been mapped by several investigators, and the rearrangement of these areas has been followed during primitive streak formation.

Gräper's (1929) conception of the disposition of presumptive material from a period preceding egg-laying to the stage of the fully formed streak is shown in Fig. 66-A<sub>1</sub> to A<sub>4</sub>, inclusive. It will be noted that the first of these diagrams represents the prospective hypoblast as a crescent-shaped area lying posteriorly, in conformity with Gräper's acceptance of invagination as the mechanism establishing this layer. Anterior to the crescent of future endoderm is a small area that is to become chorda. The center of the field is occupied by the circular medullary primordium, which is flanked on either side by presumptive mesoderm, of "unknown extent," according to this author. Areas that will later enter into some of the somites are indicated, the material for the most anterior somite being farthest caudal at this time. The second diagram gives the disposition of material at the moment the formative movements (designated by arrows) begin. As the third diagram shows, these movements supposedly reverse not only the order of the future somites but also the relative position of somite and lateral plate mesoderm, so that the latter is now the more medial. Gräper considered the floor of the primitive groove to be composed of endoderm and regarded the streak as an open blastopore; the streak, therefore, is represented in this diagram as endoderm. Much of the chorda material, as well as that for the prechordal mesoderm, has disappeared under the prospective medullary material and is not shown. In the fourth diagram one may see the invaginated mesoderm beginning to spread laterally from the anterior end of the streak. This end of the streak is now surrounded (superficially) by chordal material that was pulled toward the midline as the mesoderm invaginated.

Figures 66-B<sub>1</sub> to B<sub>4</sub>, inclusive, give the presumptive areas as visualized by Wetzel (1929), the first diagram corresponding chronologically to Gräper's second. Within the field of the future amnion and epidermal ectoderm is drawn the zone that will give rise to the medullary plate (white) and the primitive streak (stippled). This entire presumptive zone is considerably smaller than that represented by Gräper. The neural material is located



most centrally, in fair agreement with Gräper, but the future node, which Wetzel regarded as the indifferent, common source of the chorda and the floor of the neural tube, is placed well away from the periphery. The arrangement of prospective mesoderm is much different. The lateral plate mesoderm is restricted to a very small, crescentic region at the extreme

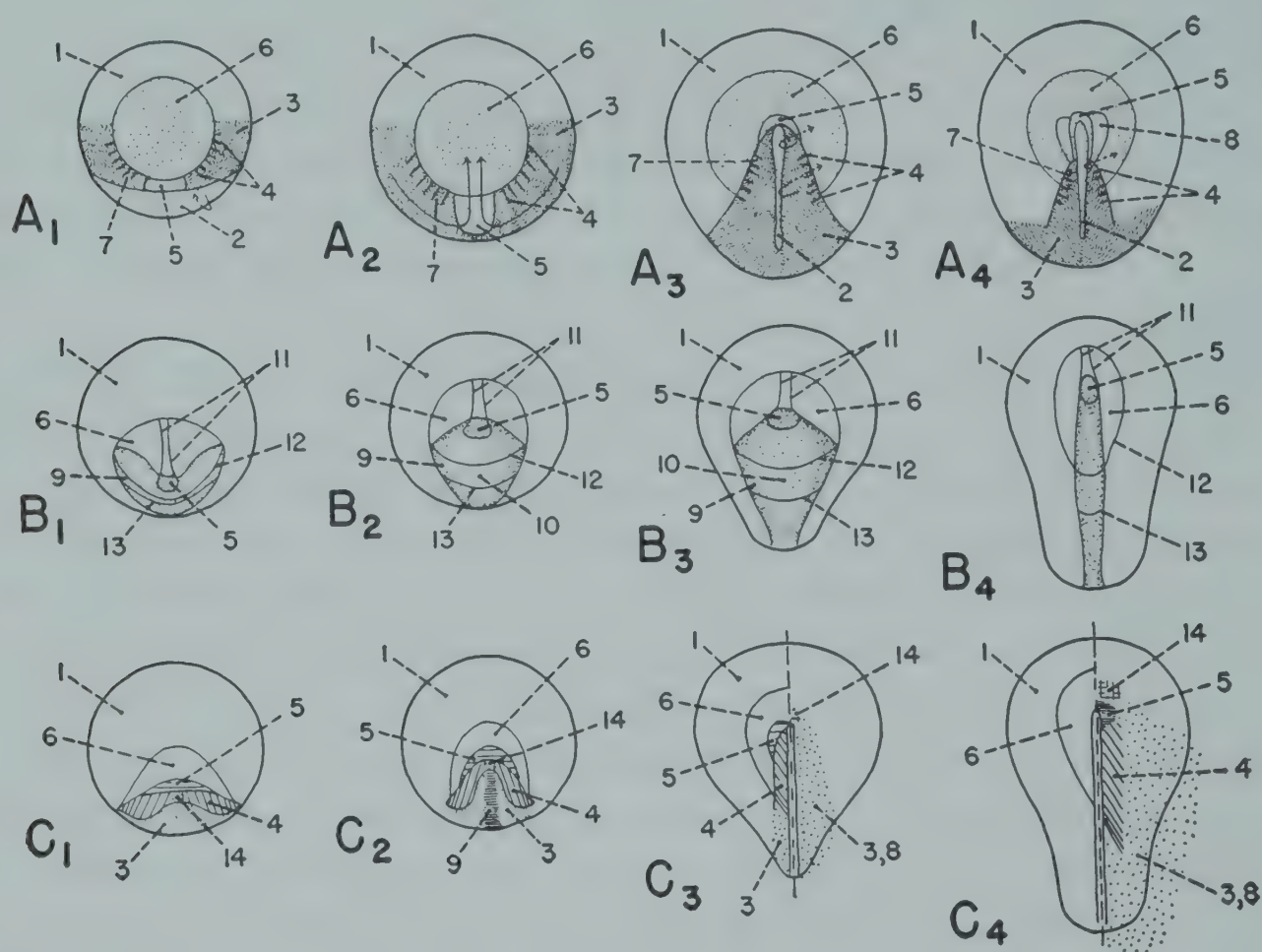


Fig. 66. Diagrams to show three different conceptions of the disposition of presumptive areas in the area pellucida of the chick blastoderm before and during the formation of the primitive streak. (Redrawn with modifications after Gräper, 1929; Wetzel, 1929; Pasteels, 1937.)

A<sub>1</sub>–A<sub>4</sub>, as proposed by Gräper (1929); B<sub>1</sub>–B<sub>4</sub>, as proposed by Wetzel (1929) showing no invaginated material; C<sub>1</sub>–C<sub>4</sub>, as proposed by Pasteels (1937). (In C<sub>1</sub> and C<sub>4</sub>, the left half of the diagram represents superficial material of the blastoderm, the right half invaginated material.)

1, epithelial ectoderm; 2, endoderm; 3, lateral plate mesoderm; 4, somite mesoderm; 5, chorda; 6, neural material; 7, first somite; 8, invaginated mesoderm; 9, primitive streak material; 10, mesoderm that will migrate out from the streak; 11, lines surrounding the median region of the chorda and the neural tube floor; 12, posterior boundary of the neural plate (corresponding to the neural organ as far posterior as the level of the twelfth somite); 13, boundary between somite and lateral plate mesoderm; 14, prechordal (pre-axial) mesoderm.

posterior border. Immediately forward lies the somitic mesoderm, with the future somites ranged in a direction perpendicular to that shown by Gräper. Most important, a large portion of the region devoted to mesoderm in Gräper's plan is represented by Wetzel as two wings of "indifferent" material which is to become nervous system as much as mesoderm. The indifferent nature of a large portion of the blastoderm is one of Wetzel's



fundamental concepts. Accordingly, he regarded the primitive streak itself as a strand of multiplying indifferent cells, a common reproduction center, through which the mesoderm must pass before actually becoming free mesoderm. Holmdahl (1925*a*, 1939*a*) expressed much the same opinion.

The schemata of Pasteels (1937) are considerably different from those of Wetzel and are reproduced in Fig. 66-C<sub>1</sub> to C<sub>4</sub>, inclusive. It can readily be seen that the prospective embryonic area touches upon a much larger portion of the posterior border of the area pellucida in Pasteels' plan than in Wetzel's. Pasteels rejected the idea of indifferent material and stated that, on the contrary, it is possible to draw a clear line between presumptive neural system and mesoderm, because the two do not coincide topographically. He found that colored marks could be applied to the anterior end of the 24-hour primitive streak in such a way as electively to stain either chorda or nervous system. Consequently, he denied the existence of a composite nodal territory and replaced Wetzel's node region with a definite chordal area distinct from the more anterior neural floor material. On his map, presumptive chorda may be found occupying a position immediately posterior to the medullary region. Pasteels was also more specific in locating the remaining mesodermal areas. Although he placed the prospective lateral mesoderm posteriorly, he extended it much farther than Wetzel. Antero-laterally, on either side, the lateral mesoderm area is bounded by future somite material. Between the bilateral wings of somitic mesoderm and directly behind the chordal area lies a small region destined to become pre-chordal plate.

The last three diagrams presented by Wetzel and by Pasteels indicate how the shapes of the presumptive areas supposedly change as the superficial material of the blastoderm wheels toward the elongating streak. The altered orientation of the future somites is to be noted in Pasteels' diagram in the third and fourth of which (Fig. 66-C<sub>3</sub> and C<sub>4</sub>) the surface of the area pellucida is represented as having been removed on the right side to reveal the invaginated material. It is clear, in these two drawings, that the lateral and prechordal mesoderm are invaginated before the chordal material.

Rudnick (1948) proposed a revised map of the original disposition of presumptive regions on the surface of the blastoderm. This map, reproduced in Fig. 67-A, is based in part on the research of Spratt (1946) on the origin of the primitive streak. The large size of the area to be invaginated is immediately apparent. In addition, the chordal, prechordal, and somitic regions are very close to the center of the blastoderm. A wide peripheral portion of the entire posterior half of the area pellucida is designated as future extraembryonic mesoderm; the lateral plate mesoderm lies medially. (Rudnick has also added a hypothetical perspective heart area medial to the lateral mesoderm and immediately posterior to the prechordal meso-



derm.) The fundamental relationships between the presumptive areas are essentially the same in this plan as in that of Pasteels, except for the fact that the chordal and prechordal regions have been moved away from the zone of initial invagination. In other words, the prospective mesodermal areas are arranged in an order that is the reverse of their relative positions after their invagination.

In the opinion of those who believed that the primitive streak arises *in situ* by proliferation (Assheton, 1896; Kopsch, 1898, 1902, 1934a; Peebles, 1898, 1904), the material that is to be incorporated into the streak and the embryonic body is of necessity disposed in its definitive craniocaudal order in the blastoderm. Figure 67-B is a map drawn by Kopsch (1934a) illustrating this concept.

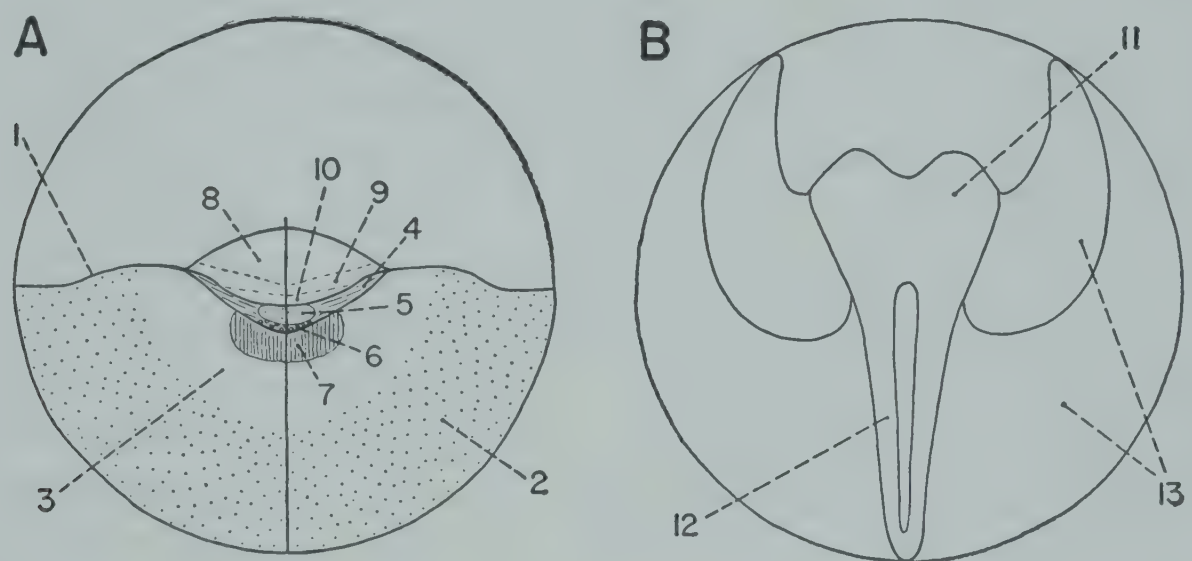


Fig. 67. Diagrams showing the original disposition of presumptive areas in the unincubated chick blastoderm, according to two different theories. (Redrawn with modifications after Kopsch, 1934a; Rudnick, 1948.)

A, as proposed by Rudnick (1948); B, as proposed by Kopsch (1934a).

1, anterior limit of material to be invaginated; 2, extraembryonic mesoderm; 3, lateral limit; 4, somites; 5, chorda; 6, prechordal mesoderm; 7, heart; 8, neural plate; 9, midbrain; 10, sinus rhomboidalis; 11, head material; 12, primitive streak material; 13, future area vasculosa (anterior horn and posterior portion).

Experimental evidence indicates that the organization of the presumptive areas in the blastoderm may not occur until a few hours after incubation begins.

### The Role of the Primitive Streak in Body Formation

It has been clear to embryologists for many years that the primitive streak is involved in the formation of the embryonic body; but the exact relationship of streak to embryo is obscure.

Many experimental attempts have been made to clarify this matter. Points on the streak or the blastoderm have been injured by such means as electrolysis, hot needles, X-rays, or have been marked with hairs or vital stains; and sections of the blastoderm have been isolated by incisions and

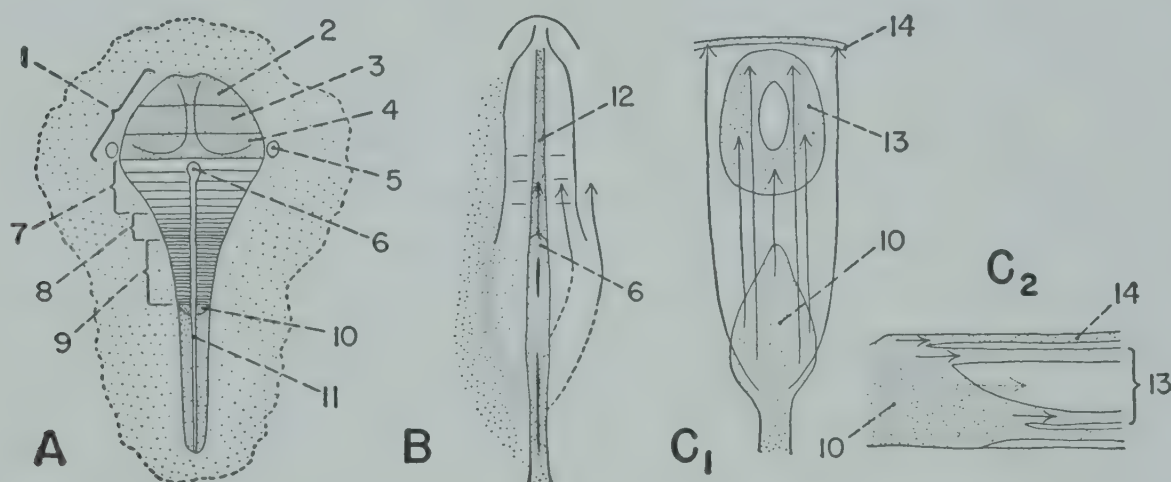


either left *in situ* or lifted out of their original location and grown elsewhere by a number of techniques. In general, the conclusions reached fall into two classes. On the one hand, the evidence has been taken as indicating a point-for-point anteroposterior correspondence between the streak and the embryo; and, on the other hand, it has been argued that such a correspondence does not exist and that the relationship of the streak to the body is indirect and fairly complex. Balfour (1873*b*) went so far as to state that "the primitive groove in the chick . . . disappears without entering directly into the formation of any part of the future animal."

The theory that various levels of the streak correspond to the same levels along the embryonic axis was evolved principally by Assheton (1896), Peebles (1898, 1904), and Kopsch (1898, 1902, 1927*b*, 1934*a*, 1934*b*, 1942)—the authors who held the view that the primitive streak arises *in situ* by proliferation. Assheton, who marked the blastoderm with sable hairs, expressed the opinion that the streak was converted directly into that portion of the embryo which lies posterior to the first pair of somites. Peebles, after performing a series of studies that involved injuring the blastoderm with needles, was more explicit than Assheton. She stated that the portion of the area pellucida lying anterior to the primitive streak gives rise to the head of the embryo; the anterior one third of the streak produces the body from the heart to the tenth or twelfth pair of somites; the middle one third forms the trunk; and the posterior one third becomes the caudal region. Kopsch's extensive experiments with electrolytic injuries led him to conclusions that are only slightly different. He divided the definitive streak (2.0 mm. long in the chick) into fourths rather than thirds and indicated that the anterior half of the streak produces all the dorsal part of both the trunk and the tail bud, while its third quarter enters into the ventral part of the tail bud and the cloacal membrane and its fourth quarter into the ventral abdominal wall. He localized the somites along the anterior half of the streak as follows: the first six somites are derived from the level of the most anterior 0.4 mm. of the streak, the next six from the following 0.15 mm., and the thirteenth to twenty-ninth from the remaining portion, 0.45 mm. long. According to Kopsch's scheme, the prospective somites lie perpendicular to the long axis of the area pellicuda (that is, to the streak), the material for the single somites becoming progressively shorter and narrower from the cranial to the caudal end of the streak. Figure 68-A reproduces Kopsch's diagram of the position and extent of prospective areas at the stage of the definitive streak. Kopsch (1934*b*) more or less summarized his view on the relationship of the streak to the embryo when he stated that the differentiation of the body starts at the cranial end of the streak and proceeds caudally; in other words, there is a progressive transformation of streak into embryo, from the anterior end backward, without any migration of the streak to the rear.



In the opinion of Wetzel (1932, 1936), the "indifferent" primitive streak resembles a blastema containing in a common matrix the materials of the entire embryo. He stated that the central part of a cross section of the body (that is, chorda, neural floor) is derived from the node, whereas the lateral parts of the neural tube and most of the somites are produced by the antero-medial part of the streak. The remainder of the somites, together with the lateral plate mesoderm and the epithelial ectoderm, develop from the posterior one quarter of the streak. In effect, his statements mean that the node corresponds to the axial parts of the body and progressively more posterior regions to progressively more lateral portions of the embryo. Figure 68-B



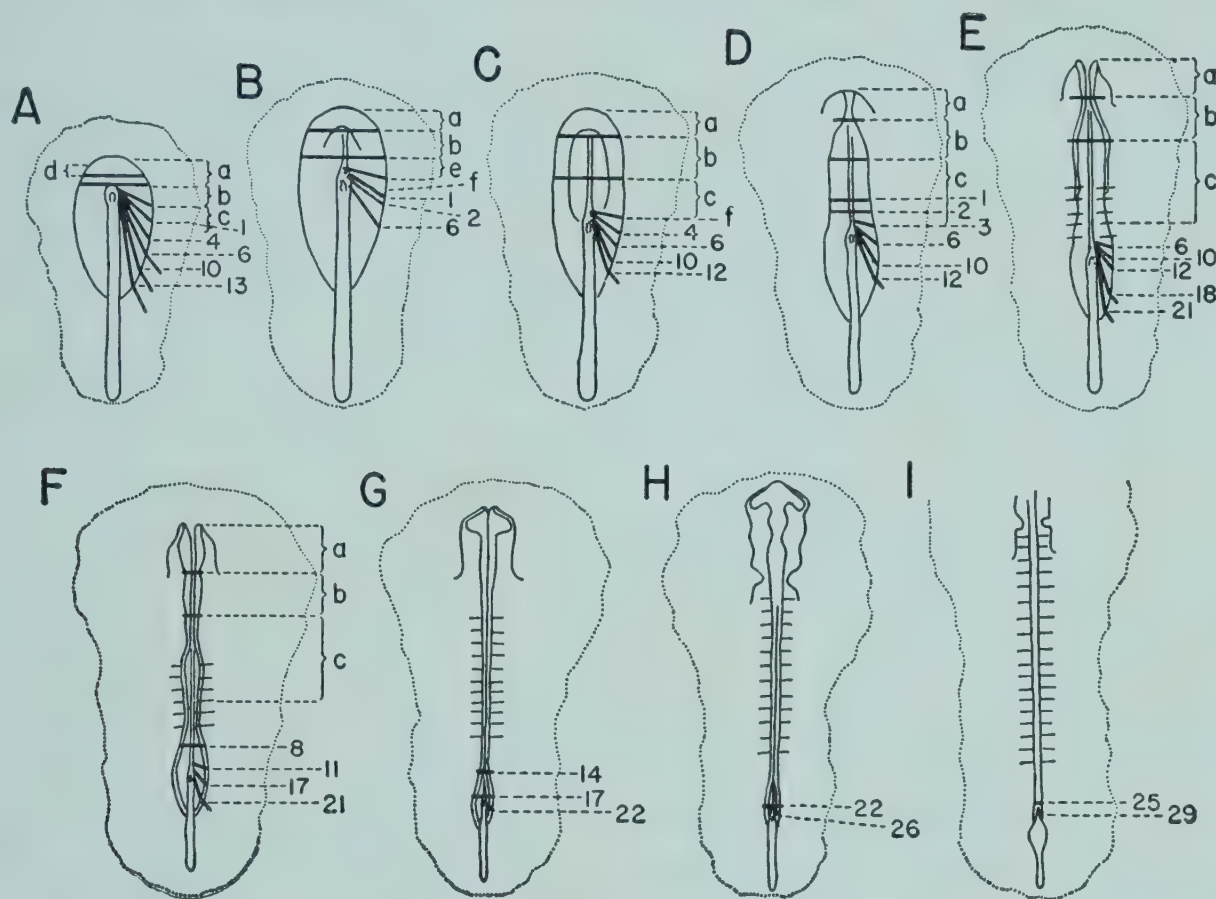
**Fig. 68.** Diagrams illustrating two concepts of the relationship of the primitive streak and the end bud to the body of a chick embryo. (Redrawn with modifications from Wetzel, 1932, 1936; Kopsch, 1934a, 1942.)

A, the blastoderm at the definitive primitive streak stage, as visualized by Kopsch (1934a, 1942); B, the origin of material of the embryonic body from various antero-posterior levels of the primitive streak, as proposed by Wetzel (1932); C<sub>1</sub>, C<sub>2</sub>, the formation of the posterior part of the neural tube from the tail bud, according to the concept of Wetzel (1936). In C<sub>1</sub>, the upper diagram is a cross section of the neural tube and superficial ectoderm and the lower diagram represents a surface view of the tail bud; C<sub>2</sub>, shows a sagittal section through the tail bud and the posterior end of the neural tube. The arrows show the direction of the cell movements.

1, brain; 2, forebrain; 3, midbrain; 4, hindbrain; 5, otic placode; 6, primitive node or pit; 7, somites 1 to 6; 8, somites 7 to 12; 9, somites 13 to 30; 10, tail bud (dorsal part); 11, cloacal membrane; 12, floor of neural tube; 13, neural tube; 14, surface ectoderm.

explains these relationships. In the end bud stage, when the neural tube is closed, it is dorsal parts of the neural tube, rather than lateral parts, that are formed from relatively posterior regions in the end bud (Fig. 68-C<sub>1</sub> and C<sub>2</sub>). Wetzel's general theory of body formation during the period when the node is regressing and the streak becoming shorter is best explained by his own diagrams, which are shown here in Fig. 69-A to I. These diagrams represent surface views. In Fig. 69-A, the oval line around the anterior half of the definitive streak shows the size and shape of the medullary plate, and the diagonal lines crossing it indicate levels corresponding more or less accurately to various future somites. Unlike Kopsch, who placed the entire

head primordium anterior to the node, Wetzel found that only the fore-brain anlage is cranial to the streak. Study of the remaining diagrams of Wetzel's series reveals how the diagonally arranged material swings into a transverse position as the forward end of the streak travels past it toward the rear. As Pasteels (1937) expressed it, the newly laid down chorda material, following in the wake of the streak, cuts through or "cleaves" the bilaterally disposed somite areas, which are thereby pivoted from the oblique position and ranged perpendicular to the chorda. As far as the



*Fig. 69.* A series of diagrams illustrating a theory (Wetzel's) of body formation during the regression of the primitive streak. (Redrawn with modifications after Wetzel, 1936.)

A, definitive primitive streak stage; B, head-process stage; C, late head-process stage; D, head fold stage; E, 5-somite stage; F, 6-somite stage; G, 11-somite stage; H, 12-somite stage; I, 18-somite stage, after the formation of the end bud (the anterior end of the blastoderm is omitted).

*a*, forebrain; *b*, midbrain; *c*, hindbrain; *d*, most anterior part of forebrain; *e*, anterior one third of hindbrain; *f*, otic vesicle. The somites are indicated by number.

change in the position of somite material is concerned, the theory fits well with the experimental findings, for, as indicated previously, it has been found that the lateral portions of the area pellucida move backward more slowly than the axial region.

A second point that emerges from inspection of Wetzel's drawings is less compatible with the results of investigations made at a later date. It will be observed that the most posterior diagonal line denotes a somite of increasingly higher number from one stage to the next; thus, in Fig. 69-B, the sixth is indicated, in Fig. 69-C, the twelfth, and so on, until, in Fig. 69-I, when



the end bud is present, the last line stands for the position of the twenty-ninth somite. Wetzel (1932) stated that all portions of the streak eventually become smaller because all parts are used. It is obvious that he has represented the streak as undergoing dissolution mainly from the anterior end backward, somewhat as Kopsch did. Reliable methods of research, however, have shown that the streak apparently shortens not at its anterior end, but mainly at its posterior extremity (see Fig. 63).

Opinions regarding body development during the subsequent end bud and tail bud stages also differ. Gräper (1933) assigned a small role to the tail bud, stating that it produced only an insignificant portion of the body. This portion was identified as the postanal region by Kopsch (1934*b*), who claimed that somites are progressively organized out of the unsegmented portion of the trunk immediately anterior to the tail bud and that this unsegmented portion shortens and corresponds to continually fewer somites as the embryo grows older. According to Holmdahl (1925*a*, 1939*a*, 1951), however, the end bud and tail bud, by active proliferation, evolve all of the embryo posterior to the level of the twenty-seventh somite. This portion of the embryo, therefore, is the product of "secondary" development, which is distinct from "primary" development in that organs form directly without passing through a germ layer stage. Wetzel (1936) similarly concluded that half the axial organs of the trunk are derived from the end bud. Seevers (1932) was also convinced that the end bud produces the notochord and the neural tube posterior to a level lying somewhere between the twenty-sixth and the thirtieth somite. The end bud, according to this concept, is a developmental center composed of undetermined or "indifferent" cells (Holmdahl, 1925*a*; Wetzel, 1932), that is, cells that do not possess the potency to form axial structures before being actually incorporated into them.

Pasteels (1937), however, contended that the concept of morphogenetic movements of well-defined presumptive areas is incompatible with the idea, proposed by Holmdahl and Wetzel, of secondary morphogenesis from an indifferent center. In his opinion, morphogenesis is a unitary process; the entire chorda and all the somites are the products of the node and the anterior part of the streak, and the end bud is merely a much reduced primitive streak, fully as heterogeneous as ever. It will be remembered that Spratt (1947*b*) considered the end bud to be composed of the node and the anterior part of the streak. As will also be recalled, the carbon-marking experiments performed by Gaertner (1949) indicate that virtually all increase in the length of the chick embryo after the 21-somite stage is due to elongation at the anterior boundary of the end bud. This elongation, Gaertner concluded, represents a rearrangement of the material of the end bud and therefore a continuation of the morphogenetic movements initiated many hours previously. As to the apparently enormous increase in the



size of the embryo anterior to the end bud, it has been suggested (for certain forms of life) that this increase represents not proliferative growth but growth by imbibition (*Pasteels, 1943*); this hypothesis may conceivably be applied to birds. *Holmdahl (1939a)* however, noted that *Pasteels (1937)* not only remarked on the great mitotic activity in the end bud but also regarded this activity as an indication of a change in the character of the primitive streak.

## ORGANIZERS IN AVIAN DEVELOPMENT

The avian egg, both before and after fertilization, is clearly an organized entity. As to how and why it becomes organized, and in what way its organizing principles function, or what these principles are—these are fundamental questions to which only partial answers as yet exist. Considerable information has been gathered regarding the causative factors in morphological development, but much more remains to be uncovered. The results of the most fruitful investigations have led to the formulation of the “organizer” concept of development.

From numerous experiments, mainly with amphibian eggs, it has been established that there are dominant and subordinate portions of the germ, a fact which is especially evident during the early periods of development. A dominant area has a certain, relatively inflexible function, which is to determine the course of development of other parts. Such an area may be considered an organization center. In amphibian eggs, the dorsal lip of the blastopore is an organization center controlling the formation of the embryonic axis; the dominance of this region may be shown by transplanting it to another gastrula, where it induces the appearance of a secondary embryo (*Spemann and Mangold, 1924*). The dorsal lip of the amphibian blastopore has been termed the “primary organizer,” but, as *Child (1946)* pointed out, this is something of a misnomer, organization in the egg obviously antedating the gastrula stage.

The activity of an organizer has been divided into two aspects (*Needham, Waddington, and Needham, 1934*). The first of these is induction *per se*, or “evocation,” which is primarily histogenetic in character. The second is morphogenetic, consisting of the molding of the induced tissues into definite morphological structures that are parts of a whole individual. This aspect is more truly organizational. In explanation of this phase of activity, it is postulated that the organization center is so constituted that one part of it determines one level of the embryonic axis, another part another level. In other words, the organization center is itself regionally organized, the gradient in its activity being known as the “individuation field.” The types of induction performed by grafts are frequently the result of the interaction, harmonious or otherwise, between the inductive capacity of the particular



part of the organization center that is grafted and the individualizing influence of adjacent regions of the host's organization center.

The nature of inductors is unknown. It has been shown many times that they have no species specificity. They may be chemical substances. On the basis of experimental evidence, the natural primary organizer has been variously identified as glycogen, as a steroid, and as a protein or a nucleoprotein (or something involved in the metabolism of any of these). On the other hand, many diverse chemical compounds, as well as dead or coagulated tissues, have been found to possess inducing powers. It is possible, therefore, that induction consists merely of an activation of cells, so that they release some intracellular component which is the actual inductor. The problem of the individuation field—that is, of the organization of parts into a whole—is more complex and has not been explained in a fully satisfactory manner.

In contrast to the organization center, the subordinate parts of the fertilized egg have a plastic or labile character in the beginning. Normally, they develop into certain tissues in accordance with their surroundings, but, in other surroundings, their “prospective significance” or ultimate fate conforms with that of their new location. This fact indicates that the “prospective potency” or possible fate of any of these areas is originally undetermined, flexible, and wider than its prospective significance. Eventually, under the influence of organizing or inducing principles, potencies are gradually restricted, and the destiny of each area is fixed in one single direction from which it cannot deviate. This destiny is now realized even when the area is moved from its normal site to another. These subordinate regions of the egg, however, must possess the ability, or “competence,” to react to inductors before any determination of fate can occur. Such ability apparently develops at the proper time; and it is thought that inductors of progressively higher grade appear in regular order to act on competent tissues, thus bringing about a sequence of differentiations. The successive influence of a series of inductors is conceived as being a basic process in development.

It should be noted at this point that the concepts of competence and determination are somewhat different from the theory of “embryonic segregation” through differential dichotomy (qualitative cell division), supported chiefly by Hoadley (1926*a*, 1926*b*, 1927) and Lillie (1929). According to these authors, successive generations of embryonic cells differentiate from a generalized type to more and more specific types by a process of segregation, the ability to self-differentiate when transplanted being the test of irreversible or unalterable prospective value. This theory implies that the segregation process gives rise to parts with new potentialities and also that it restricts potentiality from the general to the particular. Waddington (1932) pointed out that certain embryonic tissues are capable of self-



differentiation in a definite direction before they are restricted to developing in that direction, and that the arising of new potentialities (that is, in his terminology, the origin of competence) does not seem to involve a dichotomy, many competences probably existing and arising simultaneously.

The organizer concept differs also from the axial gradient theory. This theory relates morphological development to the physiological gradients that are present in the organism, the evolution of form, it is said, being caused by changes in gradient patterns (*Child, 1941, p. 723*). Inductors, at least during early development, are not specific chemical substances, but high gradient levels that act by changing conditions in, or activating, lower levels. The gradient patterns that are altered by inductors (that is, evocators) correspond to organizers, although regional character may depend upon the gradient level of the inductor region as well as upon that of the reacting part (*Child, 1941, pp. 502–503*). The evocation-individuation concept, in its implications, resembles the gradient theory stated in terms of specific substances rather than in terms of gradient levels.

### Organizers before Primitive Streak Formation

The discovery of an organization center in amphibian eggs (*Spemann, 1918; Spemann and Mangold, 1924*) led to a search for a similar center in the developing bird's egg. It is clear that the primitive streak, regardless of its blastoporal value, must itself be the product of prior organizing activity. There has been considerable speculation concerning the organization center of the pre-streak blastoderm.

Gräper (1929) suggested that this center is the posterior portion of the blastoderm, but he based his theory upon the probably erroneous assumption that hypoblast is invaginated in that region. Somewhat later, the dominant role of this section of the blastoderm was apparently indicated by the discovery that the posterior median quadrant of the blastoderm, when grafted to the chorioallantoic membrane, shows the same developmental capacity as the whole blastoderm (*Butler, 1935*). This portion of the germ was found, also, to be the only part that produces an embryonic axis when explanted to a blood plasma clot (*Spratt, 1942*), although it is possible that the failure of other parts to perform similarly may be due to the inhibiting effect of an unnatural environment (*Lutz, 1949*). Twisselmann (1938), upon observing that electrolytic injuries in the posterior portion of the blastoderm may lead to the development of two embryos, considered such duplications to be the result of splitting of the organization center.

Other experiments, mainly in the artificial production of polyembryony, have led to slightly different concepts of organizing principles in the early avian blastoderm. When the unincubated blastoderm is divided *in situ* into several pieces, a normal embryo may develop in each piece. Since any part



of the unincubated blastoderm can give rise to a complete individual, it follows that all parts are equipotential when the egg is laid (Lutz, 1949). It seems possible, therefore, that organizers are distributed over the entire blastoderm but are more concentrated in the posterior portion, which therefore dominates; isolation of this region by an incision removes its inhibiting influence on the remainder, and any other portion is then free to evolve an embryo independently (Wolff, 1948; Lutz, 1949; Spratt, 1952). Essentially the same concept has been stated somewhat differently, as follows: various potencies may extend widely throughout the blastoderm but are weakly determined in all areas except the posterior median quadrant (Butler, 1935).

Whatever the facts, the normal movement of material to the posterior midline indicates some type of dominance exerted by the caudal region. It is quite possible that any organizing capacity residing in the posterior part of the pre-streak blastoderm emanates from the hypoblast. Stimuli from this germ layer may be responsible for the cellular movements that form the streak and for the direction in which the streak develops. Spratt (1946) found that the streak usually, though not always, fails to appear if the hypoblast is removed. The directional influence of the hypoblast is revealed, presumably, by experiments in which the normal relationship of hypoblast to epiblast is altered. Waddington (1932, 1933a) removed the hypoblast from blastoderms with early primitive streaks and replaced it in such a way that the original longitudinal axis of the hypoblast was reversed or lay at an angle of  $90^\circ$  with that of the epiblast. Rotation of the hypoblast through a quarter-circle resulted in curvature of the anterior end of the streak toward the anterior end of the hypoblast, regardless of whether this lay to right or left. Rotation through  $180^\circ$  either inhibited the normal growth of the streak or caused the development of a secondary streak. When two streaks formed, either might disappear, but sometimes both persisted and gave rise to double monsters. The influence of the hypoblast may perhaps be inferred also from the marked lability of polarity exhibited by the anterior half of the unincubated blastoderm. Experiments, mainly on ducks' (*Anas platyrhynchos*) eggs, have shown that dividing the unincubated blastoderm *in situ* into halves by means of an incision perpendicular to the presumptive long axis may result in the development of an embryo in each half. The anterior embryo is frequently completely reversed cephalocaudally (Lutz, 1948b, 1949; Wolff, 1948). The lability of the anterior half of the blastoderm is confined chiefly to its median one third (Lutz, 1950b) and disappears between the sixth and twelfth hours of incubation (Lutz, 1950a, 1950c), or during the period when the hypoblast differentiates in the anterior portion of the area pellucida. Furthermore, if the anterior half of the blastoderm is isolated *in situ* by a transection, the primary endodermal layer may grow from the anterior border of the area pellucida posteriorly



toward the fissure, where a secondary invagination eventually sets in (*Lutz and Reyrolles, 1952*). Alteration in the direction of growth of the hypoblast may underlie the labile polarity shown by operated blastoderms.

### Organizers after Primitive Streak Formation

In birds, as in amphibians, transplantation of certain portions of one gastrula to another leads to the formation of a secondary embryo. The fact that various regions of the primitive streak have been repeatedly observed to perform inductions of neural tissue and even of whole or partial embryonic axes constitutes evidence that the streak corresponds in organizing activity to the dorsal lip of the amphibian blastopore. Certain workers have proposed the primitive node as the primary organization center in the chick (*Wetzel, 1925; Willier and Rawles, 1931b; Hunt, 1929, 1931a, 1931b, 1932; Umanski, 1931; Danchakoff, 1932a*), but others have contended that the presence of this structure is not essential in the evocation of embryonic parts of the streak (*Hoadley, 1926a, 1926b, 1926c, 1926d, 1927; Waddington, 1932, 1935; Waddington and Schmidt, 1933; Dalton, 1935; Woodside, 1937b; Grabowski, 1953*). *Abercrombie and Bellairs (1954)* have demonstrated that embryos in which the primitive node has been replaced at the definitive streak stage by a graft from the posterior third of the streak, leaving only head mesenchyme anteriorly, can develop nearly normal neural tubes.

It is in the anterior portion of the streak, however, that the inducing capacity seems to be chiefly concentrated (*Waddington, 1932, 1939*). Grafts of the middle and anterior thirds of the streak (*Waddington, 1932*), even when coagulated by heat (*Waddington, 1933c, 1934*), can induce neural grooves in the ectoderm of a host, but it is uncertain whether or not the posterior third has any inductive power (*Waddington and Schmidt, 1933*). In fact, the organizing influence of the streak apparently diminishes from anterior to posterior. Furthermore, the orientation of a grafted piece of primitive streak usually determines the orientation of the induced structures and it therefore follows that there is an anteroposterior polarization in the inducing power of the streak (*Waddington and Schmidt, 1933*). Axial determination, however, is a general property of the mesoderm, and reversal of the primitive streak *in situ* usually fails to alter it (*Abercrombie, 1950*).

It is more or less agreed that the mesoderm of the streak is the inducing factor. The capacity of the streak mesoderm to induce neural plate was clearly demonstrated when an implanted 18-hour primitive streak, lacking neural tissue, caused such tissue to form in the overlying epiblast of its host (*Waddington, 1933b*). It is quite likely that the mesoblast of the developing embryonic axis, brought into being by the inducing action of the hypoblast, in turn induces the hypoblast to form gut (*Waddington and Schmidt, 1933; Waddington, 1939*).



After the early gastrular stages, the power to induce neural plate is retained by the head-process and the sinus rhomboidalis, as well as by the neural plate itself (*Waddington, 1933b; Waddington and Schmidt, 1933*). It is uncertain whether or not the notochord also possesses this capacity, but it probably does when acting on epiblast of the proper age (*Woodside, 1937b*).

In birds, as in other forms, the organizing principle exhibits no species specificity. Chick tissues perform inductions in duck (*Anas platyrhynchos*) blastoderms and vice versa (*Waddington and Schmidt, 1933*), and pieces of chick primitive streak have shown inducing capacity when grafted into the embryonic shield of the rabbit (*Waddington, 1934*). Many of the chemical substances, including carcinogens, that have inductive effects in amphibians are similarly active in the chick blastoderm (*Abercrombie, 1939*).

Insofar as the responding system is concerned, the individuation field of the host blastoderm exerts an influence on the polarity and the regional character of tissues induced by grafted primitive streaks, and also on the differentiation of the grafted tissues. The effect of the host individuation field is greatest near the embryonic axis and diminishes toward the edges of the area pellucida. When placed some distance from the host axis, a graft retains a certain degree of independence; the developmental course of induced tissues is then determined by both graft and host individuation fields. On the other hand, when pieces of the primitive streak are implanted directly beneath the primitive streak, the host streak becomes the controlling factor. It may reverse the polarity of the grafted streak, and it may also impose on the graft the character of the region of implantation (*Abercrombie and Waddington, 1937*).

During the primitive streak stage, all parts of the epiblast, including the epiblast of the area opaca, are competent to form neural plate (*Waddington, 1934*). In fact, it appears that this competence exists during and possibly before short, broad primitive streak stages, which antedate the normal time of formation of the neural plate (*Woodside, 1937b*). Although the neural plate becomes irreversibly determined during the head-process (*Umanski, 1931*), the remainder of the epiblast retains its competence to form induced neural tissue until the neural folds appear. This competence does not vanish suddenly, but gradually decreases with increasing age (*Woodside, 1937b*).

Not only the epiblast of primitive streak blastoderms but also the presumptive axial or lateral plate mesoderm can be transformed into neural tissue if placed beneath the primitive streak (*Abercrombie, 1937*). On the other hand, presumptive ectoderm may be incorporated into the primitive streak as mesoderm if it is grafted into the midst of the streak, rather than beneath it (*Waddington and Taylor, 1937*). The specificity of the germ



layers, once a tenet of embryologists, thus appears to be considerably less rigid than formerly believed.

## ORGAN-FORMING POTENCIES IN THE AVIAN BLASTODERM

Many attempts have been made to determine which regions of the blastoderm can give rise to various organs—that is, to locate areas of organ-forming potency. This problem has been attacked principally by removing small pieces (usually containing all germ layers) from the blastoderm and growing them elsewhere. Although morphogenesis is not ordinarily normal under such conditions, histogenesis proceeds satisfactorily.

It should be pointed out, however, that differentiation of recognizable organ tissue in an explant from a specific region of the blastoderm at a certain period does not necessarily establish that portion of the blastoderm as the normal point of origin of the organ or organs. As mentioned previously, it appears that potencies are originally widespread and become continually more restricted, until a certain definite primordium of each organ is irrevocably established. It must also be remembered that an excised sector of blastoderm is not subject to the interplay of forces, both mechanical and physiological, operating in the intact germ; that it is, on the other hand, subject to new and usually inhibitory mechanical influences; and that it may be subject to organizers emanating from the host upon which it is grown, if it is a graft.

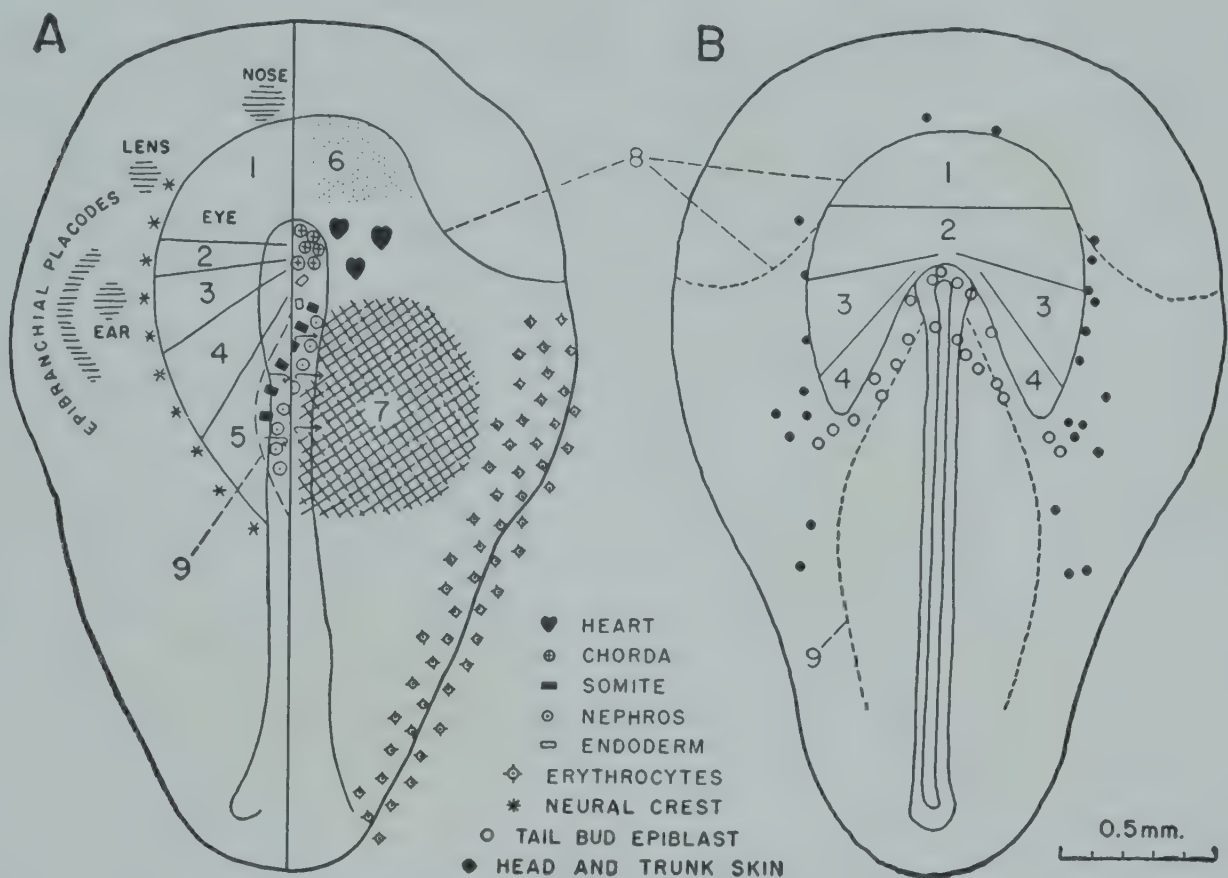
The entire unincubated blastoderm, grafted to the chorioallantoic membrane of the 9-day chick embryo, can differentiate practically all characteristic embryonic tissues (without undergoing organogenesis) and thus demonstrates the universality of its potentialities (*Butler, 1935*). Pluripotency appears to be especially concentrated in the posterior median quadrant of the blastoderm. This portion, when similarly grafted, forms as many types of tissue as the whole germ, although it will not realize its capacities if it is divided, especially in the midline. The anterior three fourths of the blastoderm form only a few tissues when grafted, notably gut, heart, and liver (*Butler, 1935*). It is conceivable that the posterolateral movement of cells plays a part in the localization of organ-forming potencies within the streak-forming posterior quadrant. We have seen, however, that the remaining portions of the unincubated germ possess wider potentialities than can be demonstrated in grafts. The differentiation of tissues in grafts of the posterior part may possibly represent reconstitution, occurring in the inherently most active region, which behaves as a whole (*Butler, 1935*).

With the formation and development of the primitive streak, the organ-specific areas undergo displacements. Mainly because of technical difficulties, it has been possible to trace the movements of only a few areas of organ-forming potency from the beginning. The location of a larger



number of areas has been determined in the definitive primitive streak and head-process stages.

Recent experiments by Spratt (1955) demonstrate that notochord and somite-forming cells realize their prospective fates only if continuous with their respective centers of differentiation. The notochord-forming center is apparently localized in the node at definitive streak and later stages, with a somite-forming center in the mesoderm on either side.



**Fig. 70.** The distribution of some ectodermal and mesodermal organ-forming potencies and prospective areas in the chick blastoderm at the definitive primitive streak stage. (Redrawn with modifications A, after Rudnick, 1944; B, after Spratt, 1952.)

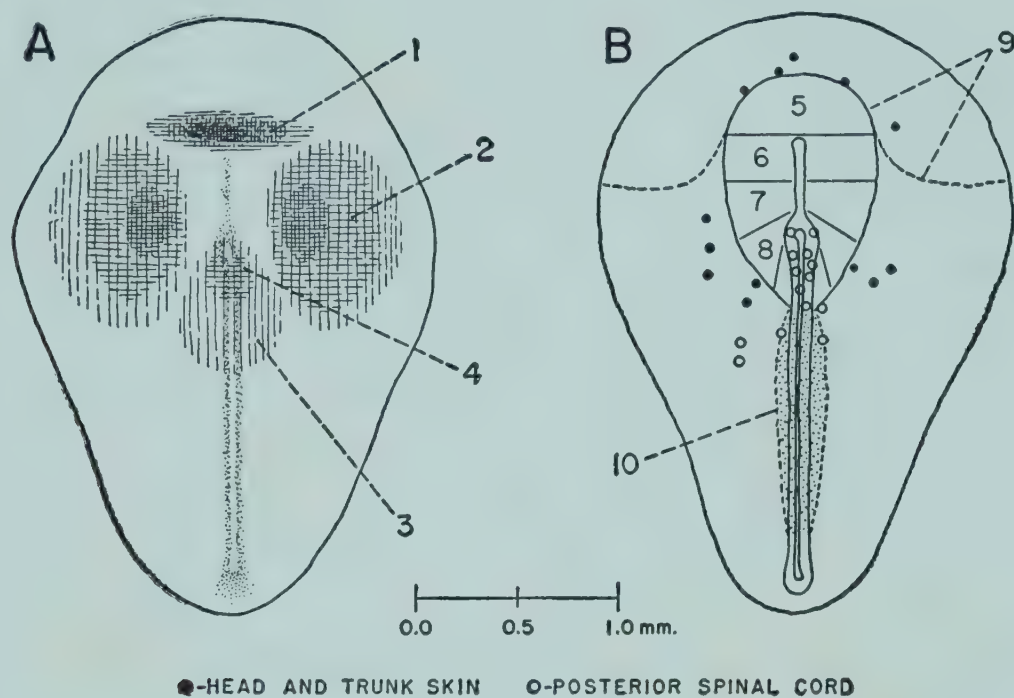
A, map showing superficial material on the left and invaginated material on the right; B, map showing the size and shape of the neural plate and its spatial relationship to prospective head and trunk epidermis, mesoderm, and material of the posterior spinal cord (that is, tail bud epiblast). Both in scale.

1, forebrain; 2, midbrain; 3, hindbrain; 4, spinal cord; 5, sinus rhomboidalis; 6, head mesoderm; 7, lateral plate mesoderm; 8, line indicating anterior boundary of invaginated mesoderm; 9, lateral boundary of uninvasinated mesoderm.

Heart-forming cells have been found in tissue cultures of peripheral areas of the unincubated chick blastoderm (Olivo, 1928e). Before incubation (Butler, 1935) and during the first 4 to 9 hours after its start (Spratt, 1942), they appear to be widespread throughout the germ. After the short primitive streak forms, they begin to concentrate in the posterior half of the area pellucida (Spratt, 1942). At this time, they are apparently just outside of the quadrant containing the streak. Somewhat later, when the blastoderm is pyriform and the primitive groove has formed, the potential heart area is closer to the streak (Rudnick, 1938c). At the definitive streak stage (Fig. 70-A), heart potency has been found to exist in the blastoderm



at levels extending from the node (*Hunt, 1931a, 1932; Rudnick, 1938a*) to the middle of the streak (*Dalton, 1935*). With the formation of the head-process, it becomes clear that the prospective heart region is situated bilaterally (*Willier and Rawles, 1935; Rawles, 1936*), consisting in actuality of two areas. On either side of the midline, these areas extend anteriorly almost to the tip of the head-process and posteriorly to a point about 0.4 mm. behind the primitive pit. Laterally, they reach almost to the edge of the area opaca. Within each area there is a gradient in developmental



**Fig. 71.** Some organ-forming areas of the chick blastoderm in the head-process stage. (Redrawn with modifications A, after *Willier and Rawles, 1935; Rawles, 1943*; B, after *Spratt, 1952*.)

A, the position and shape of the eye, heart, mesonephros, and gonad-adrenal areas, with the intensity of developmental potency indicated by the density of shading; B, the position and extent of the area destined to form the central nervous system. Both in scale.

1, eye-forming area; 2, heart-forming area; 3, mesonephros-forming area; 4, gonad- and adrenal-forming area; 5, forebrain; 6, midbrain; 7, hindbrain; 8, spinal cord; 9, anterior boundary of invaginated mesoderm; 10, lateral boundary of uninvaginated mesoderm.

potency, the capacity for differentiation into heart being greatest medially (*Rawles, 1943*). Figure 71-A gives the approximate size and location of the heart primordia at the head-process stage of development. Liver-forming areas practically coincide with heart-specific regions (*Hunt, 1931a, 1932; Butler, 1935; Willier and Rawles, 1935; Rawles, 1936*).

Erythroblasts, appearing in explants from the anterior as well as the posterior portion of the short primitive streak blastoderm (*Rudnick, 1938c*), are more or less restricted to the latter region at the definitive streak (cf. Fig. 70-A) and head-process stages (*Rudnick, 1938a*).

Cells destined to form tissue of the central nervous system seem to be localized in the anterior half of the so-called embryonic shield of the pre-streak blastoderm (*Spratt, 1952*). At the short primitive streak stage,



prospective neural tube material is found about 0.3 mm. anterior and lateral to the posterior median quadrant of the blastoderm, according to Rudnick (1938c). In the definitive primitive streak blastoderm, the neural plate extends 0.33 mm. (Rudnick, 1938b) or 0.4 mm. posterior, 0.55 mm. anterior, and 0.45 mm. lateral to the primitive pit (Spratt, 1947a, 1952). Carbon-marking experiments (Spratt, 1952) have revealed that it is more or less horseshoe-shaped (Fig. 70-B). Its oblique posterior edges are bordered by a narrow zone of epiblastic material that will be incorporated into the tail bud to form the posterior spinal cord. Between this zone and the primitive streak there is a small amount of uninvasinated mesoderm. The anterior boundary of the neural plate coincides with that of the invaginated mesoderm. The boundary between forebrain and midbrain areas is about 0.2 mm. anterior to the node, whereas at earlier streak stages the forebrain area is found at the node level. The neural plate, therefore, elongates in the region between the forebrain level and the primitive node. This elongation continues during the regression of the streak, as shown by the disposition of the prospective neural areas during the medium head-process stage (Fig. 71-B). Hindbrain and midbrain areas, as well as the forebrain area, now lie anterior to the node. The continued invagination of superficial material has brought the posterior horns, or wings, of the neural plate into contiguity with the primitive streak. The neural plate is slightly narrower because of the formation of early neural folds; also, it is shorter posterior to the node, this portion now measuring only 0.2 to 0.3 mm.

The capacity to produce feather pigment (melanin) is largely confined to the posterior half of the unincubated or pre-streak blastoderm (Rawles, 1940), whereas in the definitive streak blastoderm it is present in the node and in a surrounding area reaching 0.2 to 0.3 mm. in every direction from the primitive pit (Rawles and Willier, 1939; Eastlick, 1940; Rawles, 1940). Later, the region capable of producing pigment extends 0.3 mm. on each side of the head-process and the node, 0.1 mm. anterior to the tip of the process, and 0.4 mm. posterior to the pit. Still later, after 1 to 8 somites have formed, the capacity to form pigment is found in the neural tube, the brain wall, the node, and regions in the immediate vicinity of the node (not more than 0.3 mm. lateral and posterior to it). Eventually, with the appearance of the neural crest (see Chapter 4), cells with pigment-forming potency exist only in the dorsal half of the neural tube (Ris, 1941) and finally are confined to the neural crest itself (Dorris, 1936, 1938a, 1938b, 1939), whence they migrate peripherally (Eastlick, 1938). Pigment-forming areas therefore exemplify the gradual restriction of potency until a definite primordium is formed (Rawles, 1940).

Eye-forming potencies have also been traced and, like others, are widespread before incubation. Butler (1935) found that both pigmented and



sensory layers of the retina could be derived from anterior as well as posterior portions of the unincubated blastoderm. Between the fourth and the ninth hour of incubation, it becomes impossible to obtain retinal pigment from the posterior one fourth of the blastoderm (*Spratt, 1942*). After formation of the primitive streak, retinal potencies become localized at levels anterior to the middle of the streak and anterior to its cephalic extremity (*Hunt, 1931a; Dalton, 1935; Spratt, 1942*). At the definitive primitive streak stage, eye-forming potency is found in a single elliptical area lying transversely across the midline at the anterior end of the streak. This area extends about 0.06 mm. anterior, 0.02 mm. posterior, and 0.2 mm. lateral to the primitive pit (*Clarke, 1936*). The ectoderm that will form the lens lies lateral to the eye-forming region and outside of the neural plate (cf. Fig. 70-A). In the head-process blastoderm, the eye-forming area is still elliptical but is now found at the tip of the head-process (cf. Fig. 71-A). It extends about 0.05 mm. anterior and 0.1 mm. posterior to the anterior end of the notochord and 0.2 mm. to either side (*Willier and Rawles, 1935; Clarke, 1936; Rawles, 1936*). Investigation at both definitive streak and head-process stages has revealed that eye-forming cells appear to be more strongly concentrated in the posterior portion of this area (*Spratt, 1940*).

It may be noted that differentiation capacity in these experiments is expressed by all three germ layers comprising the blastoderm. Formation of the eye primordium from neurectoderm in normal development is presumably an inductive process.

The area of thyroid-forming potency, found in the posterior half of the unincubated blastoderm (*Butler, 1935*), migrates to the level of the node at the definitive streak stage, to extend not more than 0.3 mm. anterior to the node (*Hunt, 1932*). In the head-process stage, this potency reaches from about 0.18 mm. anterior to the end of the process back to a level somewhere between 0.26 and 0.4 mm. anterior to the primitive pit (*Rawles, 1936*). Median as well as lateral portions of the blastoderm appear to possess thyroid-forming capacity in both head-process (*Rawles, 1936*) and primitive streak stages (*Rudnick, 1930*); but, by the time the head fold has formed, it is possible that the thyroid primordium has divided into two bilateral areas (*Rudnick, 1932*).

Before incubation, both anterior and posterior halves of the blastoderm possess the ability to differentiate into gut, although esophagus forms from the posterior half only (*Butler, 1935*). In the stage of the late primitive streak, the region just behind the pit can be a source of organized gut (*Rudnick and Rawles, 1937*). In the definitive primitive streak and head-process stages, the capacity to form organized stomach is found in the area immediately around the pit (*Rudnick and Rawles, 1937; Hunt, 1932*), apparently intervening between the pit and the gut-forming cells.



In both definitive primitive streak and head-process blastoderms (cf. Fig. 70-A; Fig. 71-A), mesonephros can be formed by grafts of tissue extending from just anterior to the node to a point lying about halfway (or somewhat less) down the streak (*Dalton, 1935; Willier and Rawles, 1935; Rawles, 1936*). Mesonephros-forming potency is highest in the midline and falls off abruptly to the right, gradually to the left (*Willier and Rawles, 1935*). Anteroposteriorly, the region of maximum developmental capacity at the head-process stage (cf. Fig. 71-A) lies somewhere between the pit and a point 0.28 to 0.4 mm. posterior to it (*Rawles, 1936*). It has been said that the node level of the blastoderm still possesses the ability to differentiate mesonephros at the 12-somite stage (*Hunt, 1931a*).

Investigators are agreed that the area of adrenal-forming potency coincides with the mesonephros-forming area (*Hunt, 1931a, 1932*), except for the fact that adrenal is not found anterior to the primitive pit (cf. Fig. 71-A), does not extend so far posteriorly (*Hunt, 1932; Willier and Rawles, 1935*), and is confined to the median region (*Rawles, 1936*).

Transplantation experiments indicate that the capacity to form amnion probably coincides in extent with the lateral mesoderm at the head-process stage but is most pronounced in the median posterior portion of the area pellucida. From the latter region, amnion-forming potency diminishes gradually along the lateral boundaries of the area pellucida, and is thus concentrated in a horseshoe-shaped area (*Kumé, 1951*). This conclusion is based on the assumption that the lymph vesicles frequently observed in chorioallantoic grafts represent amnion, in reality.

It is of interest to add that the appearance of certain tissue-specific antigens antedates the formation of the corresponding organ primordia. Definitive primitive streak and head-process blastoderms explanted to media containing antiserum for chicken heart, brain, or spleen undergo disorganization characterized by loss of gross structural features and by cell clumping. Culture in antiheart or antispleen serum affects mesodermal elements and leaves the nervous system intact, though abnormal; culture in antibrain serum affects chiefly nervous tissues. In certain concentrations, the difference between the antispleen and the antiheart serums is shown by the fact that blastoderms grown in the former develop pulsating hearts, while those grown in the latter do not (*Ebert, 1950, 1952*).

## REGIONAL DIFFERENTIALS DURING EARLY DEVELOPMENT

Embryonic development does not take place at a uniform rate throughout the entire blastoderm. Certain portions of the embryo are centers of growth and differentiation, where metabolism and all life processes are



more intense than elsewhere. With the evolution of the embryo, different regions in succession become the focal points of activity.

### Electrical Phenomena

It is well known that electrical phenomena are characteristic of life in both plants and animals. In the opinion of some scientists, electrodynamic fields detectable in living organisms are not by-products of metabolic activity but are held to be the irreducible organizers of the physical and chemical constituents of living things (*Burr and Hovland, 1937*).

#### *Bio-Electric Potential Differences*

The existence of a difference in electric potential between one part of a tissue or cell and another, or between a tissue or cell and the suspending medium, is a common manifestation of life. It is not surprising, therefore, to find that bio-electric potential differences exist in the incubating egg.

In the first place, the cells of the blastoderm develop a charge that is negative to the nonliving part of the egg. Differences in bio-electric potential can be detected even through the shell of the intact egg lying on its side, with one electrode applied to the upper surface and the other to the lower surface (*Vorontzoff and Sergievskii, 1933*). The negative pole is always found on the upper surface. To facilitate measurement, small pieces of the shell may be removed and the electrodes applied to the unbroken shell membrane beneath (*Waller, 1903*). The potential difference disclosed in this manner is that which exists between the animal and the vegetal poles of the egg, for the yolk always turns so that the blastoderm lies uppermost, close to the shell. *Vorontzoff and Sergievskii (1933)* noted that 62 per cent of intact fertile eggs exhibited potential differences (as did also about 6 per cent of infertile eggs, however).

The magnitude of the potential difference between the blastoderm and the remainder of the egg depends upon several factors. For example, it is influenced by the position of the electrodes, by the incubation age, and by various types of stimulation that may be applied to the embryo.

The lowest potential difference is found when both electrodes are at some distance from the blastoderm, as they are when measurements are taken on the intact egg in the manner already described. Somewhat higher readings are obtained when one electrode is on the blastoderm and the other on the yolk of the opened egg (*Hermann and Gendre, 1884*). The greatest difference in potential is observed when one electrode is in contact with the blastoderm and the other with the albumen (*Romanoff and Cottrell, 1939b*). This is not surprising, for an independent potential



difference exists between the albumen and the main body of the yolk (Davy, 1860; Romanoff and Cottrell, 1939b; Romanoff and Bless, 1942). The magnitude of this albumen-yolk potential difference also depends on the position of the electrodes. If one electrode remains stationary at a point on the albumen and the other is moved from the center of the blastoderm to the approximate equator of the yolk, the reading at first increases to a maximum as the electrode moves to the margin of the blastoderm and then decreases until the moving electrode reaches the equator of the yolk. As Fig. 72 shows, this gradient is accentuated during the first 24 hours of incubation (Romanoff, 1944c).

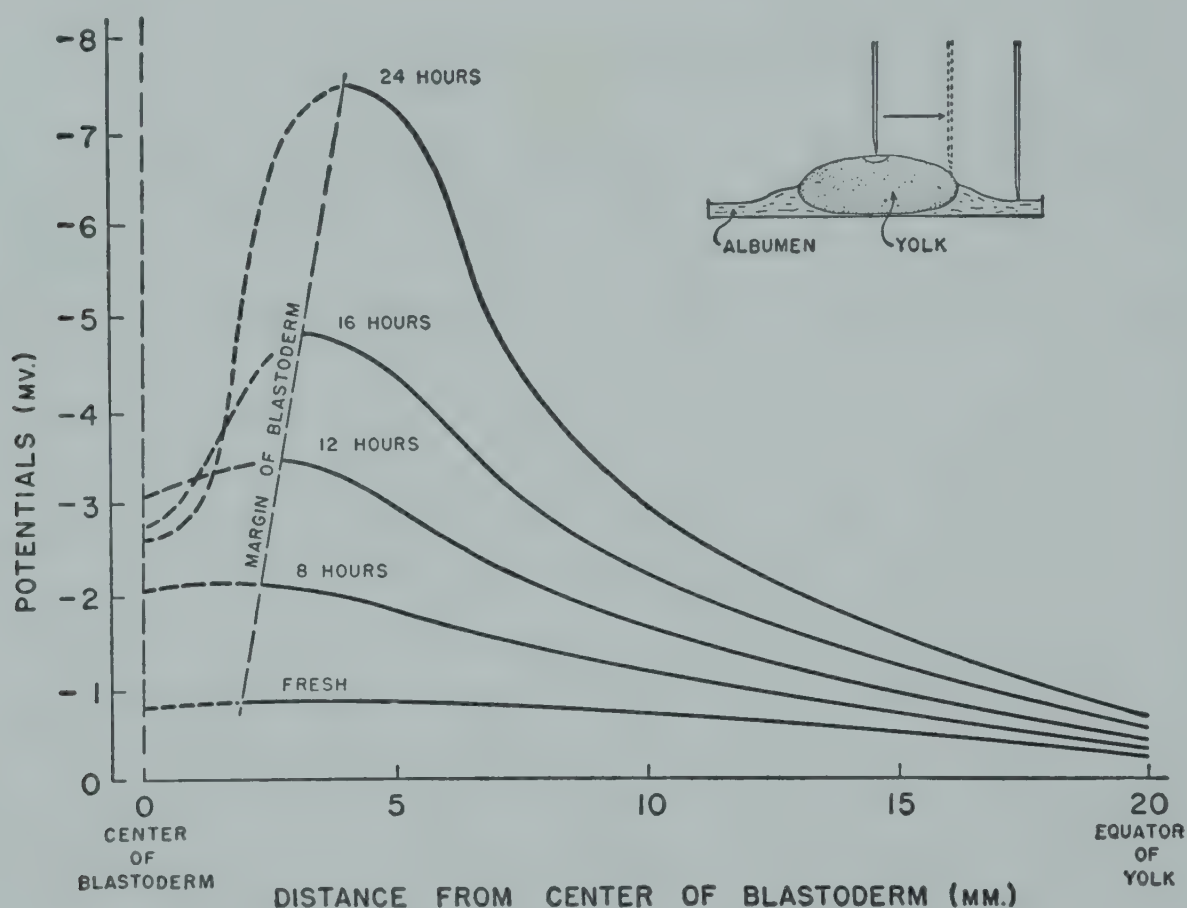


Fig. 72. Curves showing the increase during the first 24 hours of incubation of the chick in the electrical potential difference between the albumen and various points on the yolk from the center of the blastoderm outward. (Redrawn after Romanoff, 1944c.)

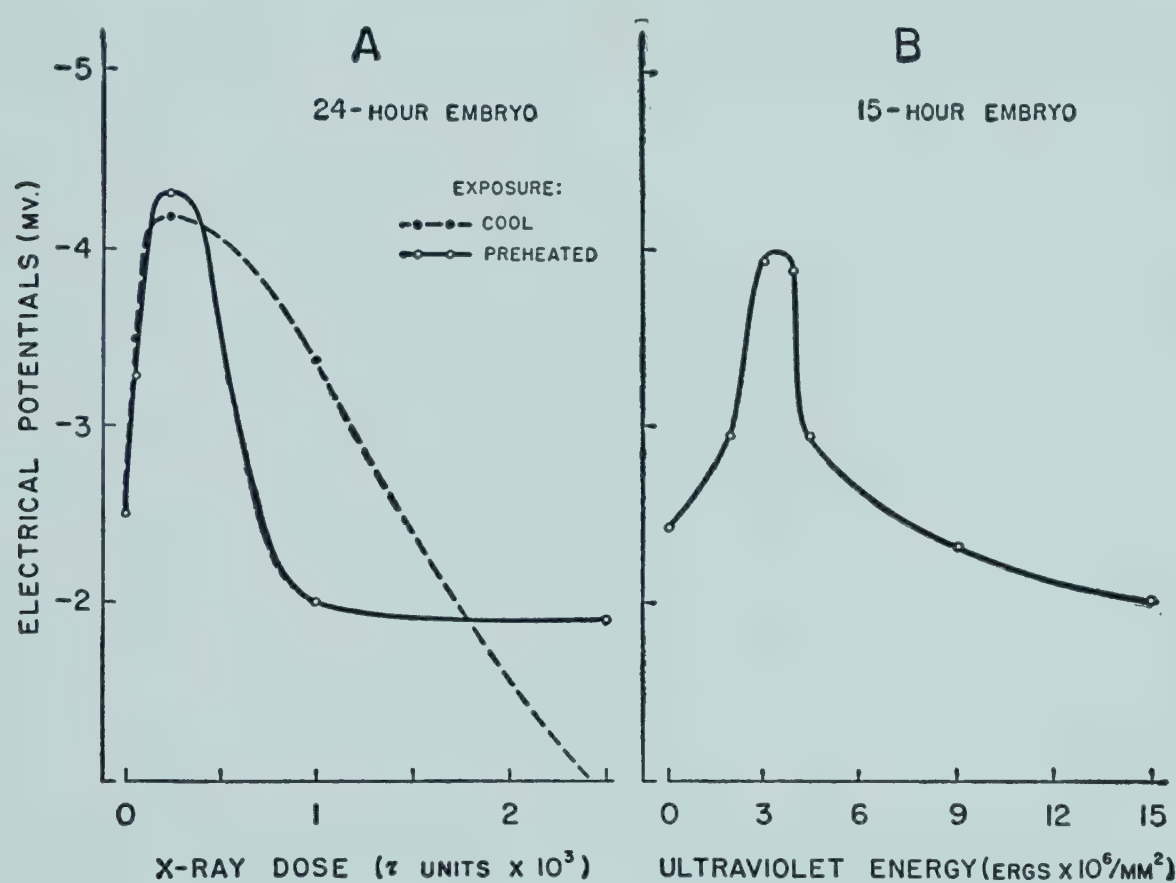
Other experiments have also shown an increase in potential difference during the first day's incubation. Vorontzoff and Sergievskii (1933) noted that the potential difference between the top and bottom surfaces of the intact egg was about 0.5 mv. at the end of 3 or 4 hours of incubation and rose to 1.0 mv. after 20 hours. Hermann and Gendre (1884), using opened eggs, reported a potential difference of 0.83 mv. between blastoderm and yolk after 8 hours and 3.55 mv. after 30 hours; Romanoff and Cottrell (1939b) found 0.8 mv. potential difference between blastoderm and albumen before incubation and 7.5 mv. at the end of 24 hours. A maximum is attained, apparently, at some time between 80 and 100 hours of incubation, and investigators are agreed that there is a subsequent decline in



potential difference between the blastoderm and the nonliving portion of the egg.

Various types of stimulating energy increase potential differences. Waller (1903), for example, found that induction shocks (applied to embryos 3 to 4.5 days old) produce responses (after-potentials) directly proportional to the electrical energy used in stimulation.

Filtered X-rays within a narrow dosage range are also effective, a dose of 250 r units exerting the maximum stimulation (*Romanoff and Bless, 1942*). When applied to eggs before incubation, this dose increases by about 75 per cent the potential difference existing between embryo and



**Fig. 73.** The potential difference between the albumen and the chick blastoderm as influenced by X-rays and by ultraviolet light. (Redrawn A, after Romanoff and Bless, 1942; B, after Romanoff, 1943c.)

A, potential difference as a function of the dosage of X-rays applied before incubation to cool and to preheated intact eggs; B, potential difference as a function of the amount of filtered ultraviolet radiant energy applied after 15 hours' incubation.

albumen at the end of 24 hours' development (Fig. 73-A). Doses above 500 r units are distinctly inhibitory. Inhibition due to radiation is more pronounced, and the range of stimulating doses is narrower, in eggs preheated before being radiated, that is, in eggs in which activity is high.

Ultraviolet light is another form of energy that may increase potential differences. Ultraviolet rays of maximum transmission in the region of 3650 Å have been found to produce an immediate rise of about 60 per cent in the potential difference between blastoderm and albumen of the 15-hour incubated egg (Fig. 73-B). The same total energy ( $3.0 \times 10^6$



ergs/mm<sup>2</sup>) with rays of about 3100 Å is only about one half as effective (Romanoff, 1943d).

A 10-minute exposure of eggs (preheated at incubation temperatures for 4 hours, or incubated 12 to 18 hours) to a high-frequency field (25 megacycles) leads to a 60 per cent increase in the potential difference between blastoderm and albumen in preheated eggs and to a 10 per cent increase in incubated eggs (Romanoff, 1946).

Finally, there is evidence that an increase in the oxygen content of the air to 75 per cent for a few hours during early development may also result in a rise in potential differences (Romanoff, unpublished).

**Gradients in Potential Difference within the Embryonic Areas.** As noted above, a lower potential difference is found between the albumen and the center of the blastoderm than between the albumen and the

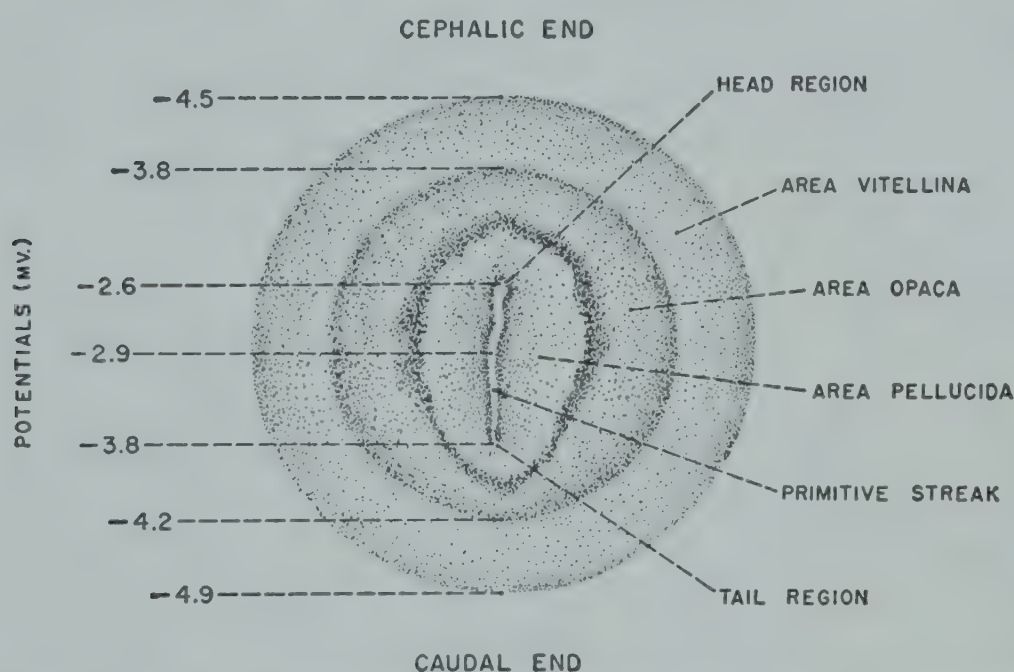


Fig. 74. The potential difference between the albumen and points along the longitudinal axis of the chick blastoderm in the primitive streak stage. (Redrawn after Romanoff, 1944c.)

margin of the blastoderm. Figure 74 shows the typical bio-electric potential differences between the albumen and the longitudinal axis of a 16-hour chick blastoderm in the definitive primitive streak stage. It will be noted that all potentials are negative to the albumen potential, and that the largest negative values are found at the margin of the blastoderm, the smallest along the primitive streak. Furthermore, slightly larger negative values are observed at the caudal end of the streak and at the caudal margin of the blastoderm than in the cephalic portions of these structures. These findings apparently indicate that electrical activity during the primitive streak stage is highest around the margin of the blastoderm, especially the caudal margin, and at the caudal end of the primitive streak. Studies by Burr and Hovland (1937) provide evidence that there is a negative cephalocaudal potential gradient after the first day of incubation (probably in association with the development of the nervous system).



### *High-Frequency Conductivity*

Experiments with intact, new-laid eggs placed in an inductance coil and exposed to a high-frequency electric current indicate that the average conductivity of fertile eggs is lower than that of infertile eggs (*Romanoff and Cottrell, 1939a*). Further investigation has shown that this difference is primarily due to a pronounced decrease in the conductivity of the albumen during the very early stages of embryonic development, together with a slight increase in the conductivity of the yolk during the same period (*Romanoff and Frank, 1941*).

### *Metabolic Gradients*

It is undoubtedly true that the regions of relatively great developmental—or differentiation—activity in the avian blastoderm are also the regions of highest metabolic rate. Variations in metabolic activity have been demonstrated in a number of ways.

Hyman (1927*a*) found that susceptibility to lethal solutions (potassium cyanide, ammonium hydroxide, and sodium hydroxide, all in isotonic salt solution) varies considerably in different portions of the chick blastoderm. In primitive streak stages, disintegration caused by these agents starts at the anterior end of the streak and proceeds caudad. With the formation of the head fold, a double gradient in susceptibility becomes apparent, for disintegration begins at the anterior end of the primitive streak and progresses posteriorly; the medullary plate is then attacked in the same manner. As the neural folds arise, they acquire high susceptibility and likewise disintegrate from anterior to posterior. In early somite stages, disintegration of the embryo begins at the node and proceeds forward and backward simultaneously, and the neural folds become most susceptible midway down their length, in the region of incipient fusion. After a considerable portion of the neural tube has closed, the disintegration gradient of the nervous system shifts again to the anteroposterior type. Hyman regarded these regional differences in susceptibility to killing agents as indicating differences in rates of cellular activity, the most active regions being the first to die and disintegrate.

Hinrichs (1927), using lethal doses of ultraviolet, also noted that there is a simple anteroposterior gradient in susceptibility during early stages, followed by a double gradient in later stages. Sublethal doses revealed the presence of this double gradient through regional variation in the incidence of abnormalities caused by irradiation.

Buchanan (1926) tested the inhibitory effects of hydrocyanic acid on the development of the chick embryo. The areas which he found to be most easily disturbed correspond roughly to those designated by Hyman (1927*a*) as the most active. Since hydrocyanic acid depresses metabolic



processes, especially biological oxidations, he concluded that oxidative activity varies as follows in different regions of the incubating egg: (1) it is more rapid at the margin of the blastoderm than in the area pellucida (excluding the embryo); (2) it is greater in the embryo than in the extra-embryonic tissues; (3) during the closure of the neural folds, it is relatively high in the crests of the folds; (4) it is highest of all in the head region of the embryo. Buchanan's failure to note a double gradient is explained by Hinrichs (1927) as probably due to recovery of the posterior portion of the embryo over the relatively long period of exposure to the inhibitory agent.

Spratt (1950a) observed that chick blastoderms explanted in head-process and early somite stages to a nonnutrient medium start to degenerate in 2 to 4 hours. In this instance also, a double gradient of degeneration is apparent, one center at the node and the other at the cephalic end of the embryonic axis. An identical pattern of degeneration was seen when certain concentrations of metabolic inhibitors (sodium monoiodoacetate and sodium fluoride) were added to a nutrient substrate. This pattern clearly corresponds to the pattern of differentiation activity, which Spratt related to enzymatic activity and especially to the activity of the enzymes of oxidative metabolism and glycolysis (see Chapter 10).

Oxidative activity in the chick embryo was studied by Rulon (1935), who stained blastoderms with the oxidized form of Janus green, sealed them from the air, and observed the progress of reduction of the dye. Rulon observed that reduction begins in primitive streak blastoderms at the periphery of the area pellucida, occurs next in the node, and then spreads out laterally from the streak and posteriorly along its length. In head-process and somite stages, the various regions of the embryo proper reduce the dye in essentially the same order in which Hyman (1927a) reported that they disintegrate. The data of Miller (1941) and of Tomita and Yasuzumi (1941) agree with those of Rulon. The latter pair of investigators also reported a gradient in the reduction of toluene blue, brilliant cresyl blue, and methylene blue. The regions where reduction is most rapid coincide with the regions containing the largest concentrations of glutathione, a hydrogen receptor, and with the regions where the labile oxidase reaction is most marked.

Philips (1941a, 1942) measured the respiration of different parts of the chick blastoderm (by means of the Cartesian diver microrespirometer) and was unable to detect any significant variations in the consumption of oxygen. He criticized the work of Rulon (1935) and Miller (1941), whose results he considered inconclusive. According to Philips, the regions in which Rulon and Miller found the highest rate of Janus green reduction are the same regions in which the number of cells per unit area is the greatest. By virtue of this fact, Philips contended that these regions may



be expected to reduce dye before others which contain fewer cells per unit area. However, he also pointed out the fact that oxygen consumption is only one of many respiratory activities and suggested the possibility that embryonic activities may have been suspended under the conditions of his own experiment.

Fraser (1956) has observed that cytochrome *c* activity is greater on a per cell basis in the mesoderm of the primitive streak than in the epiblast. A sharp pattern of differential reduction is described (Spratt, 1955) as centering in the node and particularly in the newly forming chorda cells.

### *Histochemical Changes*

Using histochemical methods, Jacobson (1938*a*, 1938*b*) studied the changes in the distribution of glycogen in the avian blastoderm from a time previous to incubation until after the formation of the primitive streak. Before the appearance of hypoblast, the cells at the periphery of the blastoderm are richer in glycogen than those in the central area. Once they differentiate, all hypoblast cells (with the exception of those at the rim of the area pellucida) are almost entirely free of this substance. In primitive streak stages, glycogen is present in all regions of the epiblast, including that of the area opaca, but it is almost completely lost by the cells that invaginate through the streak.

The same investigator (Jacobson, 1938*b*) has also reported on the lipid content of the various regions of the primitive streak blastoderm. All germinal layers are rich in fatty substances, which probably are sterols and not, apparently, phosphatides or cerebrosides. In early streak stages, the lipoids in the epiblastic cells increase in amount with greater proximity to the streak and are most abundant in the streak itself, where they diminish in concentration from anterior to posterior. As the mesoblast moves away from the streak, its cells suffer a progressive loss in lipoids. Jacobson related this mediolateral gradient to a metabolic change, since the lipoids in the head-process are unaffected by the elongation of this structure.

Clues to the rate of protein synthesis in various parts of the blastoderm have been obtained by procedures that reveal the distribution of sulfhydryl groups, nucleoproteins, and ribonucleic acids. Brachet (1940) noted that the hypoblast of the unincubated chick blastoderm gives the nitroprusside reaction for sulfhydryl groups less markedly than the epiblast and that nuclei give a stronger reaction than cytoplasm. The primitive streak, after its appearance, is always the darkest region, especially at the node. The observations of Buño (1951) are in essential agreement, but this author noted further that sulfhydryl groups are present in the yolk adherent to the area opaca, in the head fold, in the neural plate, and in the mesoderm adjacent to the area opaca anterior to the proamnion; the head-process gives a negative reaction. The regions of positive reaction are those where



there is a convergence of morphogenetic movements. Gallera and Oprecht (1948) studied the localization and synthesis of ribonucleic acids and found that these are present in the unincubated blastoderm only in nuclei, where they are confined to nucleoli and to a few granules adhering to the nuclear membrane. After 4.5 hours' incubation, they begin to appear around some of the superficial cells in the median and posterior regions of the blastoderm. In early primitive streak stages, the color reaction for these substances is intensified, especially in the epiblast and the streak. The color intensity of both epiblast and mesoblast diminishes rapidly toward the periphery, and there is also a dorsoventral gradient in the mesoblast. The head-process is strongly colored throughout its length. The neural plate, as it develops, gives an increasingly strong reaction, as does the bottom of the medullary groove. Eventually, the mediolateral gradient in the mesoderm disappears, and this layer is colored intensely throughout. It is especially well colored in its posterior portion, where the blood islands form. The somites, when they appear, react markedly. In somite stages, the hypoblast cells (always most weakly colored) begin to show color in their cytoplasm.

### NUTRITION OF THE EARLY CHICK EMBRYO

There have been few studies of the nutrition of the chick embryo of less than 3 days' incubation. Since yolk spheres are commonly observed within the cells of the early blastoderm, it can be inferred that this yolk material constitutes a direct source of energy for cellular metabolism. Studies of blastoderms grown in artificial media indicate, however, that the explanted embryo cannot depend solely upon this endogenous supply of nutriment.

The inability of the blastoderm to continue development in the absence of exogenous nutriment is demonstrated by its gradual degeneration when explanted (in the primitive streak or head-process stage) to a nonnutrient saline-agar substrate (Spratt, 1947c). The fact that the embryo dies very promptly on substrates purposely made toxic indicates that degeneration on a saline-agar medium is the result of starvation and not of poisoning. Addition of either yolk-albumen extract or buffered yolk extract to the nonnutrient substrate restores the capacity of the blastoderm to grow and differentiate *in vitro* for 2 or more days at an approximately normal rate and to live for several additional days.

Some indication of the role of the albumen is obtained by the failure of a nonbuffered saline-agar medium containing pure yolk extract to support the blastoderm. The albumen is apparently essential in maintaining the environment of the blastoderm at the optimum pH for development (Spratt, 1947c). The experiments of Schmidt (1937) provide evi-



dence, too, that the osmotic pressure of the egg white is of great physiological importance in the regulation of water exchange between the yolk and the embryo.

The ability of egg albumen to act also as a nutrient during the early developmental period is shown by the fact that the addition of albumen extract alone to the nonnutrient medium permits normal differentiation of the embryo. Growth, however, is somewhat less than optimal (*Spratt, 1947c*). Schmidt (1937) noted that the organic components of albumen are indispensable for the start of development.

In investigating the nutritive requirements of the early chick embryo, *Spratt (1948)* successively removed various constituents from a medium containing (in addition to buffered saline-agar) the ten essential amino acids, cysteine hydrochloride, seven vitamins of the B-complex, vitamins A and C, and glucose. This medium supported essentially normal growth, morphogenesis, and differentiation of primitive streak and head-process blastoderms. After the removal of amino acids, or of both amino acids and vitamins, only the growth process was affected adversely; morphogenesis and differentiation were not noticeably influenced. On the other hand, when glucose alone was removed from the substrate, development of the blastoderm ceased entirely, and degeneration commenced within a few hours.

From the above experiment, it may be reasoned that amino acids and vitamins are utilized during the early incubation period and that their function at this time is primarily the promotion of growth. It is also apparent that the growth process is distinct from the processes of morphogenesis and differentiation.

The most important conclusion to be drawn, however, is that glucose is absolutely indispensable in the initial stages of development. As *Needham (1925; 1931, pp. 986-999)* has emphasized, there are various clues pointing to the probability that glucose is the chief constituent of the egg metabolized by the chick embryo during the first week of incubation. Chemical analyses by *Bywaters (1913)*, *Satô (1916)*, and *Idzumi (1924)* showed a rapid decline in the free sugar content of the egg for a week or 10 days after development starts; and *Tomita (1921)* discovered that lactic acid, a product of sugar metabolism, increases sharply in the egg during this period. Schmidt (1937) also remarked on the growth-promoting effect of a glucose solution (of the same concentration as that of glucose in undiluted egg albumen) used as a medium in which to incubate intact yolks. Indirect evidence that carbohydrates initially constitute the main source of energy is provided by the results of respiration studies. In the primitive streak stage (*Needham, 1932c*) and for the first few days of incubation, the respiratory quotient of the chick embryo (*Hasselbalch, 1902; Bohr and Hasselbalch, 1903; Fukahori, 1933*) and of its tissues



(Dickens and Simer, 1930, 1931) approaches unity (Needham, 1931, pp. 702-703; 1932b), indicating the metabolism of carbohydrates predominantly. Philips (1941b) observed that the rate of oxygen consumption of early embryos in buffered Ringer solution was made constant by the addition of 0.2 per cent glucose to the medium.

The explanted chick blastoderm can utilize several sugars in addition to glucose (Spratt, 1949). These are the disaccharide, d-maltose, and three others—d-mannose, d-fructose, and d-galactose—which, like glucose, are naturally occurring hexoses. In ability to support the blastoderm, however, they are not equal. Mannose is approximately equivalent in activity to

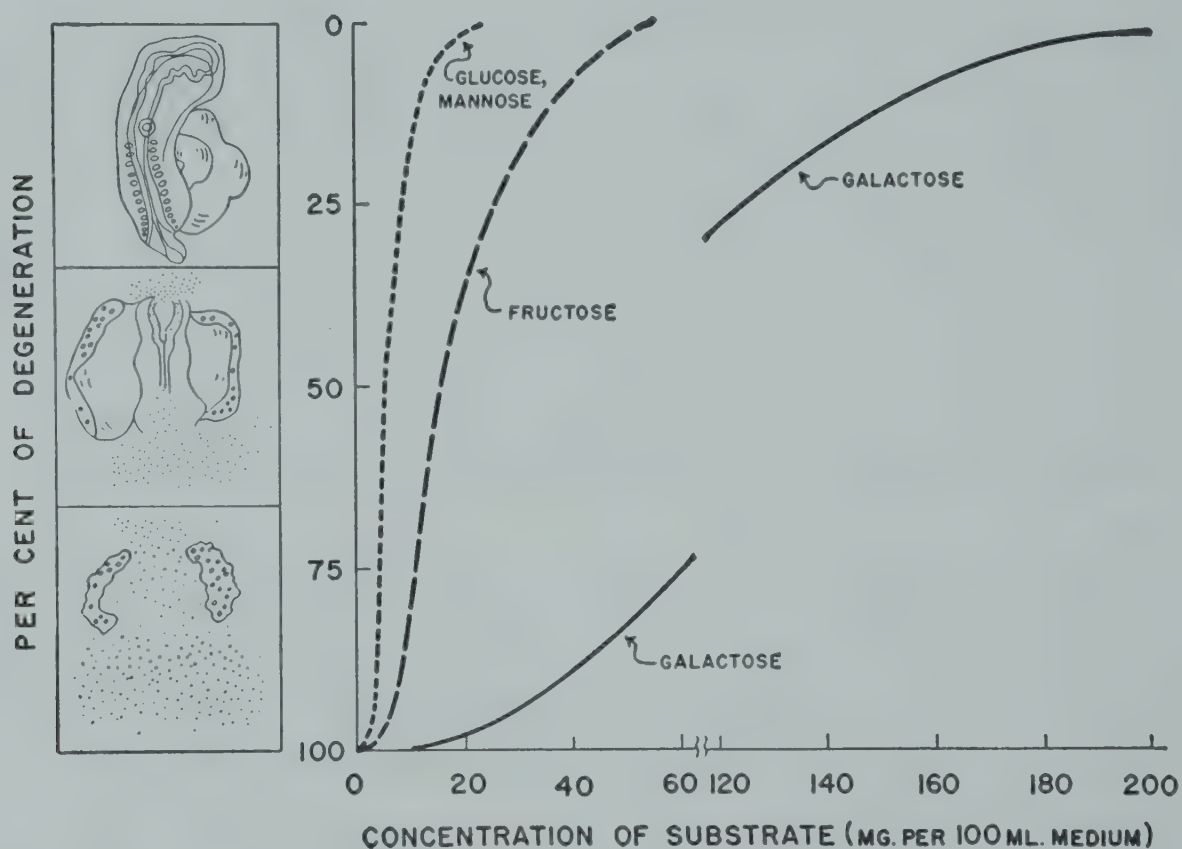


Fig. 75. The comparative ability of glucose, mannose, fructose, and galactose to support the chick embryo *in vitro*, as indicated by the relationship between their concentration and the degree of degeneration occurring in the embryo. (Drawn from the data of Spratt, 1949.)

The *Inserts* at the left represent (top to bottom) 0, 50, and 100 per cent degeneration.

glucose, 15 to 20 mg. of either sugar per 100 milliliters of medium being necessary to prevent degeneration; but 50 mg. of fructose, 200 mg. of galactose, and about 400 mg. of maltose must be used (Spratt, 1949). Figure 75 compares the effectiveness of these sugars graphically. Spratt (1949) suggested that differences in utilization of the hexoses reflect differences in the embryo's ability to phosphorylate them and to interconvert the phosphorylated intermediates for further metabolism. Since maltose was the only disaccharide utilized out of six tested, it is possible that its availability depends either on the embryo's capacity to hydrolyze it to its constituent hexoses or on the presence in the developing egg of a phosphorylase for maltose.



It has been generally believed that the free glucose of the egg is the sugar utilized during the early developmental period. However, the proteins of the yolk and the albumen contain prosthetic sugar groups, and, in several proteins, these bound carbohydrates are polysaccharides of the mannose-glucosamine type. As yet, it is unknown whether or not proteins constitute a source of carbohydrate for the embryo. Bywaters (1913) concluded that sugar was not split off ovomucoid because the carbohydrate moiety of this protein remained unchanged throughout incubation. Taylor and Schechtman (1949) grew blastoderms on media containing yolk extract that had been dialyzed, heated, and re-dialyzed to remove free sugar and to destroy enzymes which might liberate sugars, but these investigators noted that the capacity of such yolk extract media to support differentiation was retained to a considerable extent. It appears that some nondialyzable yolk constituent of large molecular size contributes to the nutrition of the embryo, and it is possible that enzymes of the blastoderm itself split sugar from this component.

Konopacka (1933) made a microchemical study of the utilization of fats and phosphoproteins during ontogenesis of the chick. In the unincubated egg, the phosphoprotein globules of the white yolk beneath the blastoderm may be seen to break up into small globules and then either to dissolve or to become vacuolized, as would occur if certain substances were being removed in preference to others. The cells of the blastoderm contain globules of both phosphoprotein and fat: After 24 hours' incubation, there are few phosphoprotein globules in the cells of the epiblast, but fat droplets are numerous in this layer and are even more abundant in the mesoblast and hypoblast. In the germ wall cells, there is active dissociation of yolk globules into phosphoproteinous and fatty portions, the phosphoproteins fragmenting, vacuolizing, and dissolving as before. Modification of the phosphoprotein globules is much more advanced in the portions of the germ wall closest to the embryo. The results of this investigation led to the conclusion that the separation of fats from the white yolk globules is followed by the breaking down of the phosphoproteins into proteins and phosphatides, which are then used for the continually increasing production of nuclein and cellular protoplasm.

### THE INFLUENCE OF ENVIRONMENT ON EARLY DEVELOPMENT

The early development of the avian embryo may be profoundly affected by environmental factors. Temperature, physical vibrations, air composition, irradiation with X-rays or ultraviolet rays, and other natural or experimentally applied influences may modify the course of embryonic evolution. The embryo is susceptible to the impact of forces such as these



throughout incubation and, in fact, before it begins, in the interval that may elapse between egg-laying and the start of incubation. The results of early external conditions may become apparent during the first 24 hours or not until much later.

### The Effect of Temperature

In the evolution of the avian embryo, the influence of environmental temperature is paramount. Below a certain thermal point, development does not proceed at all; above this point, the rate and regularity of development increase with rising temperature until an optimum is reached.

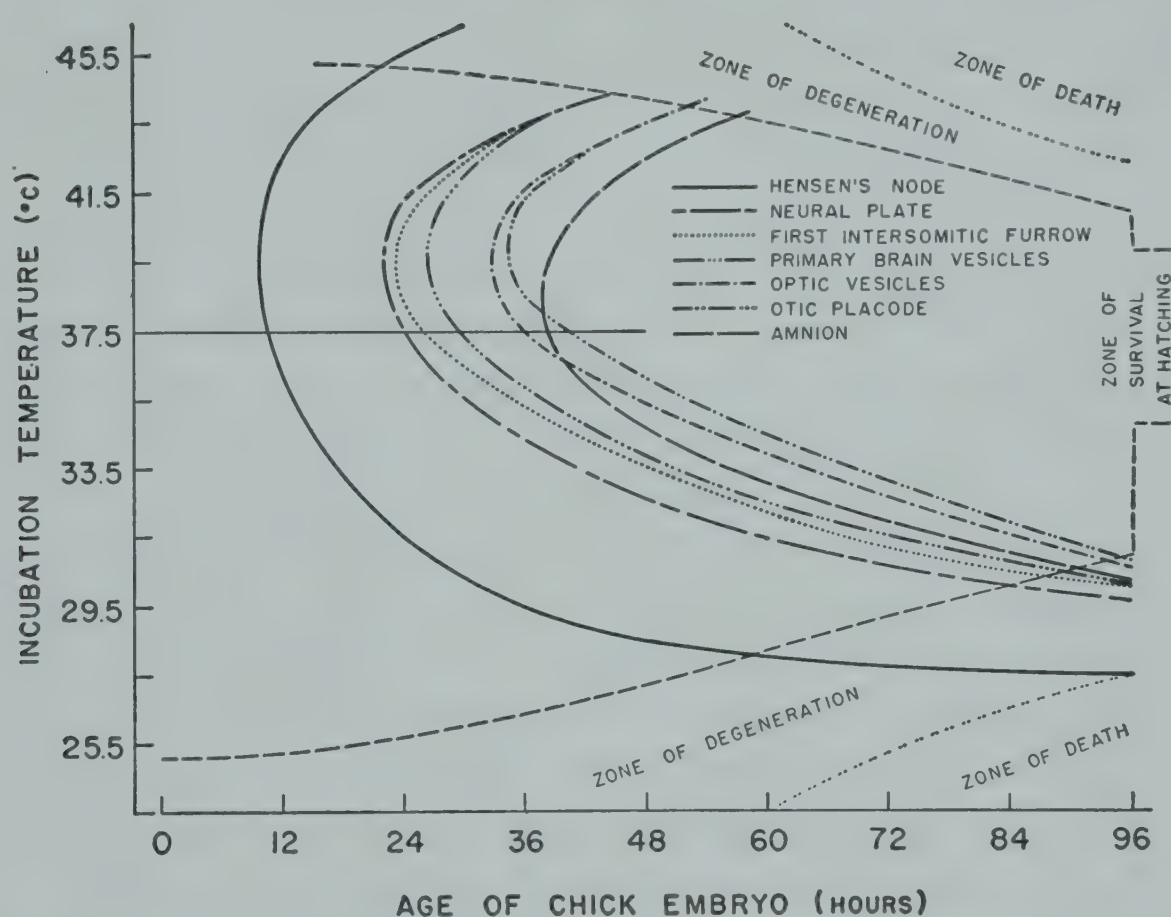


Fig. 76. Influence of incubation temperature on the time of appearance of various early structures in the chick (*Gallus gallus*) embryo. ("Zone of survival at hatching" after the data of Romanoff and Faber, 1933; Romanoff, 1936; the remaining data after Romanoff, unpublished.)

There is a maximum temperature beyond which life is almost immediately extinguished; and there is also a minimum temperature which the embryo can withstand, although this minimum does not remain constant throughout the course of incubation.

For many years, it was generally agreed that a temperature of at least 28° C. is necessary to initiate development in the laid chicken egg. This is the figure given by Dumas (1824, *p.* 121), as quoted and corroborated by Dareste (1891, *p.* 116), although Dareste (1865, 1869) had previously designated 30° C. as the minimum. However, noticeable development has been reported at 25° C. (Rauber, 1884; Harrison and Klein, 1954) and 26° C. (Köl liker, 1879, *p.* 99), and an extensive study made by Edwards



(1902) indicated that the temperature of  $20.5^{\circ}\text{C}$ . may be the physiological zero for the hen's egg. Edwards observed primitive streak formation in about 39 per cent of all eggs held for several days at temperatures from  $20.7^{\circ}\text{C}$ . to  $27^{\circ}\text{C}$ ., inclusive. He claimed that the blastoderm was enlarged in the remaining 61 per cent of the eggs, but other investigators (*Funk and Biellier, 1944*) have denied that there is any discernible development of the blastoderm at temperatures below  $27^{\circ}\text{C}$ . The investigations of Philips (1941*b*) showed no activation of respiration (which would have been revealed by an abrupt rise in oxygen consumption) at  $28^{\circ}\text{C}$ .; instead, a linear increase in oxygen consumption was found to occur over the temperature range of  $22^{\circ}$  to  $38^{\circ}\text{C}$ . Philips' findings tend to support those of Edwards (1902). According to Deuchar (1952), 3 hours at  $45^{\circ}\text{C}$ . produces abnormal development, especially at the long streak stage; and 5 hours of this treatment has marked ill effects on all embryos from pre-streak through long streak stages.

Less attention has been paid to the determination of the maximum temperature at which development is possible. Dareste (1891, *p. 118*) considered this temperature to be  $43^{\circ}\text{C}$ ., thereby disagreeing with Dumas (1824, *p. 121*), whom he quoted (*Dareste, 1891, p. 116*) as giving  $44^{\circ}\text{C}$ . or  $45^{\circ}\text{C}$ . as the maximum.

As noted by Dareste (1891, *p. 118*), the optimum temperature for the development of the chick embryo lies within the range of  $35^{\circ}\text{C}$ . to  $39^{\circ}\text{C}$ . According to Féré (1894*b*), a temperature of  $38^{\circ}\text{C}$ . is the most desirable during the first few days of incubation. In general,  $37.5^{\circ}\text{C}$ . may be considered optimal (Fig. 76).

### *The Influence of Low Temperature*

The ability of the avian blastoderm to survive at temperatures far below the level that permits development depends upon the degree of cold and the period of exposure to it. The embryo's response to chilling differs before and after the start of incubation. Furthermore, its developmental rate is related to the temperature of incubation, when this is depressed below the optimum.

**Pre-Incubation Resistance to Cold.** Under natural conditions, birds' eggs are not subjected to incubation immediately after being laid. Ordinarily, a bird lays several eggs at intervals of 1 day or more until a clutch of normal size is completed, and not until then does she start to brood. In the interim, developmental processes in the first eggs of the series may be completely in abeyance. The eggs of wild birds and many domesticated ones are exposed during the pre-incubation period to environmental temperatures determined solely by the vagaries of the climate. It is not surprising, therefore to discover that the avian blastoderm is capable of withstanding a relatively severe degree of cold for a fairly long time.



As remarked by Dareste (1891, *p.* 101), the viability of the blastoderm prior to incubation is very variable and depends upon the individuality of the egg as well as upon the influence of environment. However, within certain limits and in the absence of complicating factors, the temperature of storage is the most important single factor determining how long the embryo can live after the egg is laid. This fact has been known and put to practical use for many years, in all probability for centuries before Réaumur (1749, *vol.* 1, *p.* 144) made note of the superior hatching power of winter eggs as compared with summer eggs.

TABLE 3  
Influence of Exposure of Fertile Eggs to Low Temperature on Development and Hatchability of Chicks (*Gallus gallus*)

Temperature	Period of Exposure	Effect on Embryonic Development	References
—18.0° C.	0.5 hour *	Not killed †	Rabaud (1899)
— 9.4° C.	1.5 hours *	Not injured	Colasanti (1875) Elford (1921) Mussehl and Bancroft (1925)
— 2.8° C.	Several days	Killed ‡	Pictet (1893) Mancini (1908)
— 1.1° C.	4 nights in succession	Normal hatch	Dougherty (1926)
0.0° C.	12 hours	Normal hatch	
"	24 hours	10% reduction in hatch	Mussehl and Bancroft (1925)
"	36 hours	25% reduction in hatch	
"	48 hours	50% reduction in hatch	Mauro (1923)
"	72 hours	Destroyed in hatch	

\* The eggs were practically frozen.

† The hatched chicks showed numerous monstrosities.

‡ The subsequent development of the embryo was abnormal.

There have been many experiments designed to test the ability of the dormant chick blastoderm to withstand cold. In 1875, Colasanti found no subsequent loss of developmental capacity in eggs held for 2 hours at —7° C. and —10° C. Pictet (1893), however, claimed that the embryo is killed at —2° C. to —3° C., although able to survive at —1° C.; but he did not state for how long a period he held eggs at these temperatures. Development, although anomalous, was observed by Rabaud (1899) in eggs exposed to a temperature of —18° C. for a half hour before incubation. According to Mauro (1923), 72 hours' refrigeration at 0.5° C. is fatal, as is



30 days' storage at  $14^{\circ}\text{C}$ .; at the latter temperature, however, 40 per cent of his experimental blastoderms remained alive for 20 days. Musschl and Bancroft (1925) found that holding eggs for 18 hours at  $0^{\circ}\text{C}$ . had no detrimental effect at all and that only a few blastoderms lost their developmental capacity in eggs kept at this temperature for 36 hours (although the longer exposure appeared to reduce the vitality of some of the hatched chicks). Lipschütz and Illanes (1929) noted normal development in eggs subjected to preliminary chilling at  $-4^{\circ}\text{C}$ . and  $-6^{\circ}\text{C}$ . for 4 hours. Table 3 shows the effect of exposure of unincubated fertile eggs to various low temperatures on the further development of the embryo and hatchability of chicks.

As Moran (1925) pointed out, many of these scattered observations did not take into account the time required for the interior of the egg to reach the environmental temperature. His investigations showed that the center of a hen's egg of average size reaches the temperature of  $0.7^{\circ}\text{C}$ . in 12 hours,  $-2.9^{\circ}\text{C}$ . in 24 hours, and  $-4.6^{\circ}\text{C}$ . in 30 hours. After determining the actual time the egg's entire contents were held at these temperatures, he calculated that the maximum survival time was 9 days 9 hours at  $0.7^{\circ}\text{C}$ ., 4.5 days at  $-2.9^{\circ}\text{C}$ ., and 47 hours at  $-4.6^{\circ}\text{C}$ . These data illustrate not only the ability of the blastoderm to endure chilling, but also the direct correlation between the degree of cold and the length of time that the blastoderm remains viable. Decreasing longevity at temperatures near or below zero was explained by Moran as probably being the result of irreversible physicochemical changes in the yolk of the egg.

In addition, Moran (1925) stated that the optimum storage temperature for the maintenance of life in the blastoderm is in the vicinity of  $8^{\circ}\text{C}$ . to  $10^{\circ}\text{C}$ . He found the limiting time of storage to be 39 and 34 days at these two temperatures, respectively. Other investigators have observed that the unincubated blastoderm survives longest at  $12^{\circ}\text{C}$ . to  $14^{\circ}\text{C}$ . (Fig. 77).

*The after-effects of low pre-incubation temperatures.* In spite of the fact that the blastoderm can survive exposure to relatively severe cold prior to incubation, the course of subsequent development reveals the fact that such exposure may have a harmful effect. Rabaud (1899) found that embryogenesis ceased in the stage of the primitive streak and the early medullary groove in many of the eggs that he had subjected to a preliminary chilling at  $-18^{\circ}\text{C}$ . for 2 hours. Like Rabaud, Grodzinski (1933) noted that preliminary refrigeration resulted also in the appearance of many blastoderms without embryos on the third day of incubation. Such "anidian" blastoderms have been observed by many embryologists. Grodzinski, who investigated their causation, found that the first change to take place is the disappearance of the germ wall. In eggs cooled to  $-3^{\circ}\text{C}$ . for 1 to 6 days, no trace of the germ wall remains after 2 hours of incubation. The entire blastoderm then appears opaque and white. Almost im-



mediately, it begins to diminish in size, becoming from 20 to 50 per cent smaller. The shrinking process may consume from 5 to 7 hours. After a pause of varying length (2 to 5 hours), growth begins again. Nile blue sulfate marks on the surface of the blastoderm showed that the shrinkage of the blastoderm is caused by a centripetal movement of cells and that the resumption of growth is due to an abnormal centrifugal movement of cells in the area pellucida. Grodzinski suggested that the disappearance of the germ wall is responsible for this abnormality of cell movement.

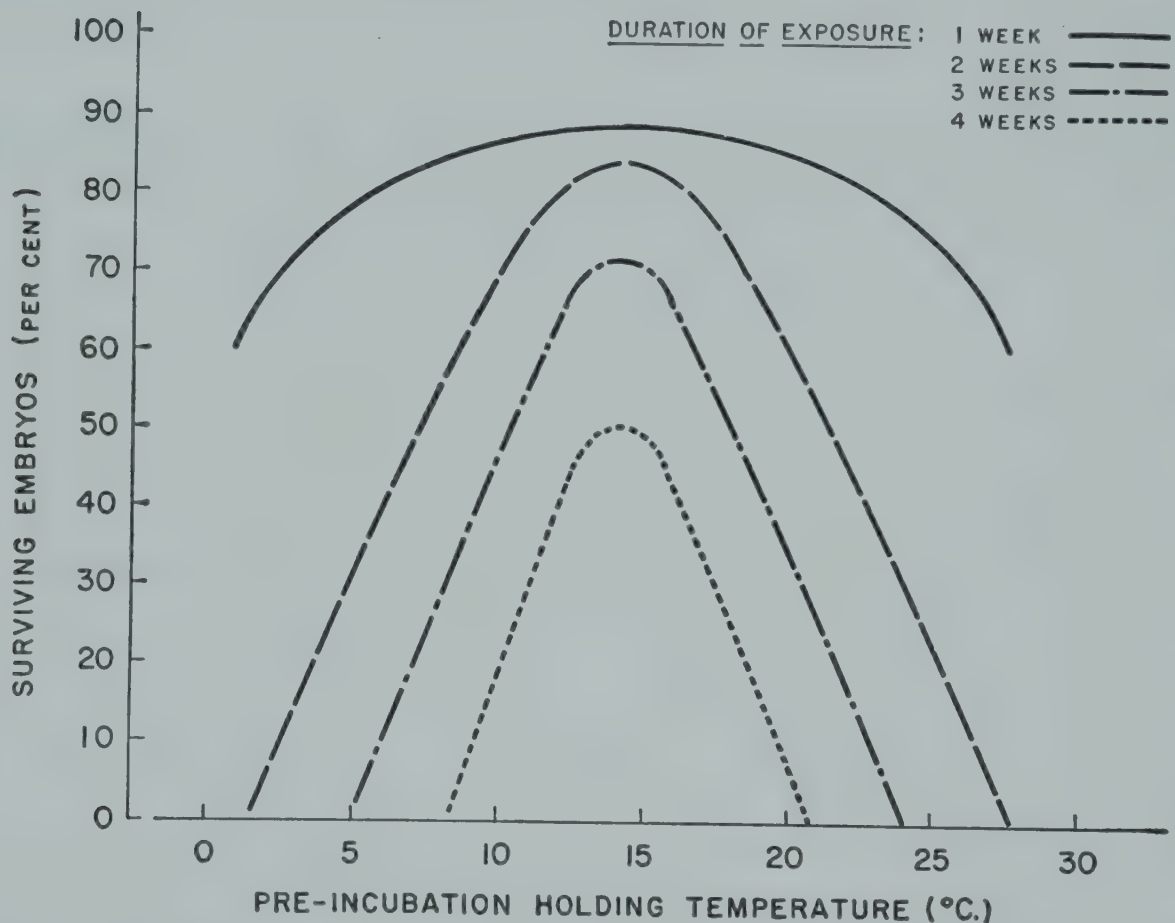


Fig. 77. Curves showing the ability of the chick blastoderm to withstand various pre-incubation storage temperatures. (Drawn from the data of Waite, 1919; Mauro, 1923; Fronda and Andres, 1932; and Scott, 1937.)

**The Effect of Low Incubation Temperatures.** After incubation begins, the blastoderm becomes more sensitive to the influence of low temperatures. In experiments designed to reveal the effects of cold, temperatures either below 28° C. or between this point and that of normal incubation have been applied both continuously and intermittently.

Many years ago, Dareste (1865) commented upon the slowness of development in the absence of sufficient heat. In 1891 (*p.* 117), he remarked that embryogenesis at 28° C. or 29° C. is extremely retarded and that it is followed by death at an early stage. He stated, in addition (*p.* 119), that delayed development at 30° C. to 34° C. also leads to death, but in more advanced stages, and added (*p.* 129) that this degree of cold, although arresting development, probably kills the embryo indirectly through its effect on functional processes.



Darrest's early observations have been borne out by subsequent investigations. Féré (1894*b*) found that 48 hours of incubation at 35° C. carries development only slightly past the 24-hour stage shown in the charts of Duval (1889). At 34° C., development after 48 hours is at the normal 16- or 18-hour level. Alsop (1919) also observed that embryos incubated 48 hours at temperatures no lower than 34.4° C. may develop only to the 24-hour stage.

These statements may be supplemented with more specific data. The size of the blastoderm, for example, clearly shows the influence of incubation temperature. The accompanying tabulation indicates how its growth during the first 24 hours is retarded by temperatures only a few degrees below normal (Romanoff, Smith, and Sullivan, 1938). Edwards (1902) gave information on the much more inhibitory effect of a lower tempera-

Incubation Temperature (° C.)	Diameter of 24-Hour Chick Blastoderm (mm.)
33.5	5.5
34.5	7.7
35.5	9.4
36.5	11.0
37.5	12.0

ture range. After 6 days of incubation at various points between 20.7° C. and 27° C., inclusive, the diameter of the blastoderm increased very little, as the table shows.

The time of appearance, size, and regression of the primitive streak also are affected by low temperatures. Although the primitive streak of the chick may form before the end of the first 12 hours of incubation at all temperatures down to 31.5° C., it does not appear until well beyond this time at lower temperatures and may even require 24 hours or more to develop at 28.5° or 27.5° C. (Romanoff, *unpublished*). Data presented

Incubation Temperature (° C.)	Diameter of 6-Day Chick Blastoderm (mm.)
20.7	4.77
21.1	4.87
21.4	5.33
25.0	6.28
27.0	7.15

by Edwards (1902) and summarized here in tabulated form indicate how the growth of the streak is delayed in the absence of sufficient heat. After 6 days' incubation at temperatures from 27° C. to 20.7° C., the streak does



not approach the length normally attained in 24 hours. Figure 78 illustrates graphically the delayed regression of the streak at three subnormal incubating temperatures, 35.5° C., 33.5° C., and 31.5° C.

Incubation Temperature (° C.)	Length of 6-Day Primitive Streak (mm.)
27.0	1.32
25.0	1.23
21.4	0.90
21.1	0.83
20.7	0.70

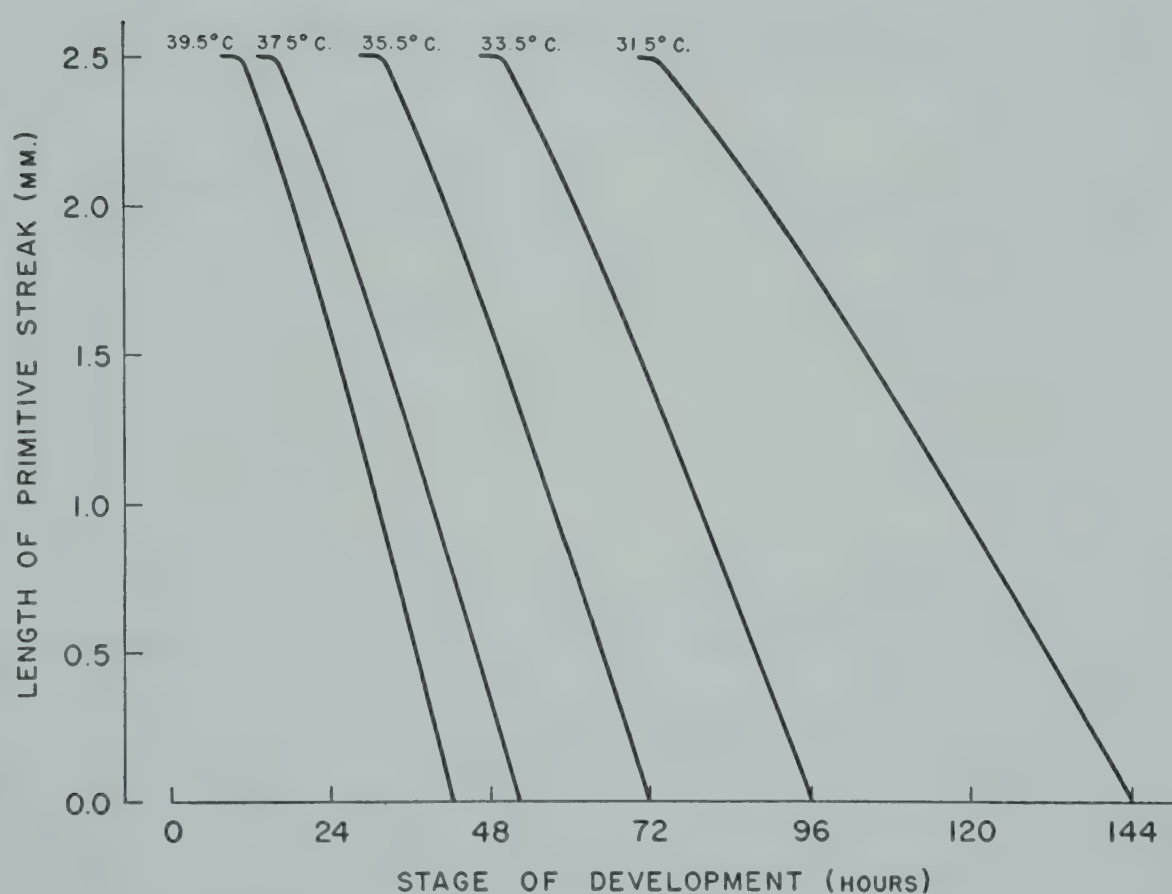


Fig. 78. The relationship between incubation temperature and the time required for complete regression of the primitive streak in the chick blastoderm. (Drawn from unpublished data of Romanoff.)

Study of other individual early structures of the chick embryo has shown that they are progressively slower to form at increasingly low temperatures (*Romanoff, unpublished*). A constant temperature of 34.5° C. considerably retards the development of Hensen's node, the notochord, and the neural groove. At 30.5° C. the node does not become apparent until well past the first 24 hours. The notochord and neural groove are delayed to the same extent at 34.5° C., and an additional 2-degree decrease may prevent their appearance for another 12 hours. The pronounced inhibition of somite formation at temperatures below 35.5° C. is indicated in Fig. 79.

The inhibition of embryonic development by lack of heat has been



demonstrated by Tazelaar (1928), who used pipes containing hot and cold water to distribute warmth unevenly to incubating eggs. Figure 80-A represents an embryo in approximately the 24-hour stage after 74 hours of incubation under a temperature gradient applied in this manner. The blastoderm was cooled anteriorly and is pear-shaped because of the retarded growth of the cranial portion. The primordial embryonic body, lying anterior to the primitive streak, is relatively short in proportion to the length of the streak, and the neural folds are poorly formed. In Fig. 80-B may be seen the results of applying the opposite temperature gradient for 72 hours. The anterior part of the embryo has developed normally, but the cooled posterior part is retarded.

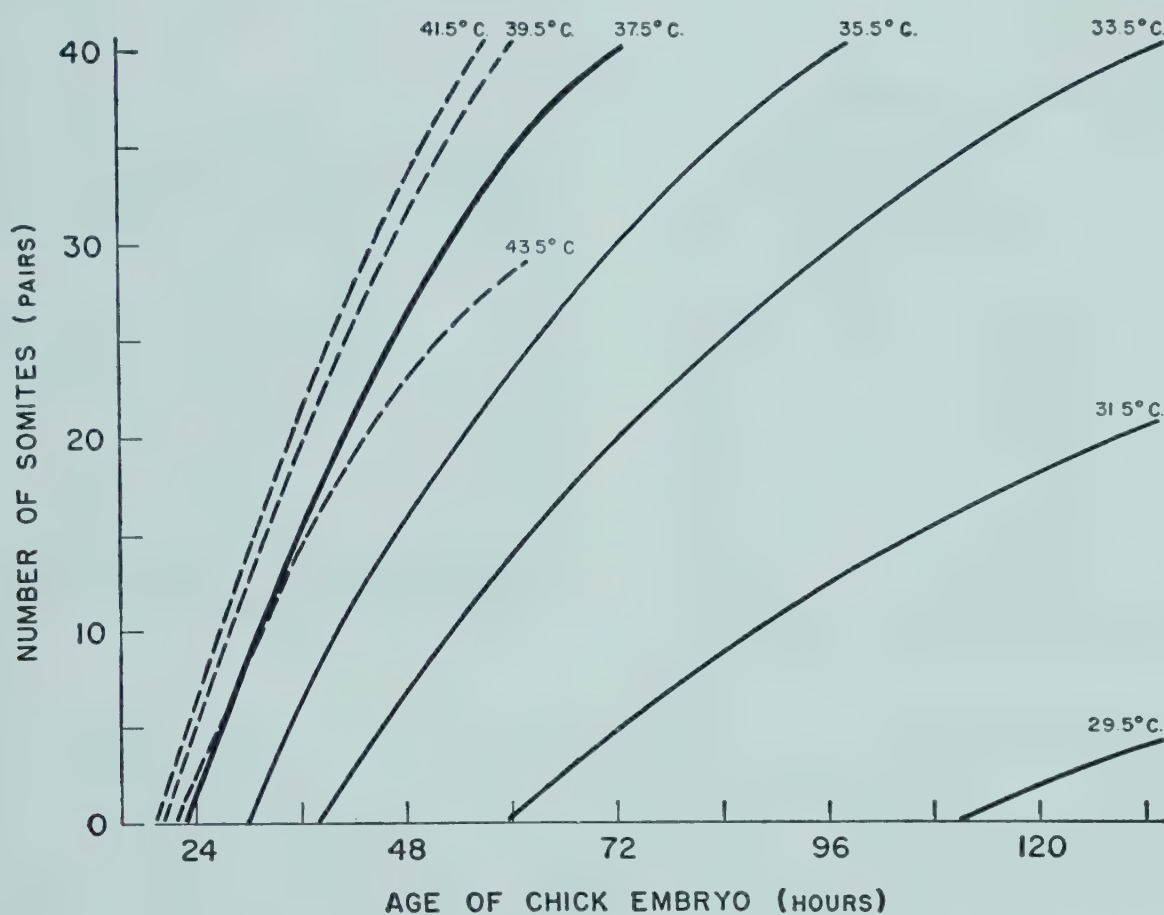


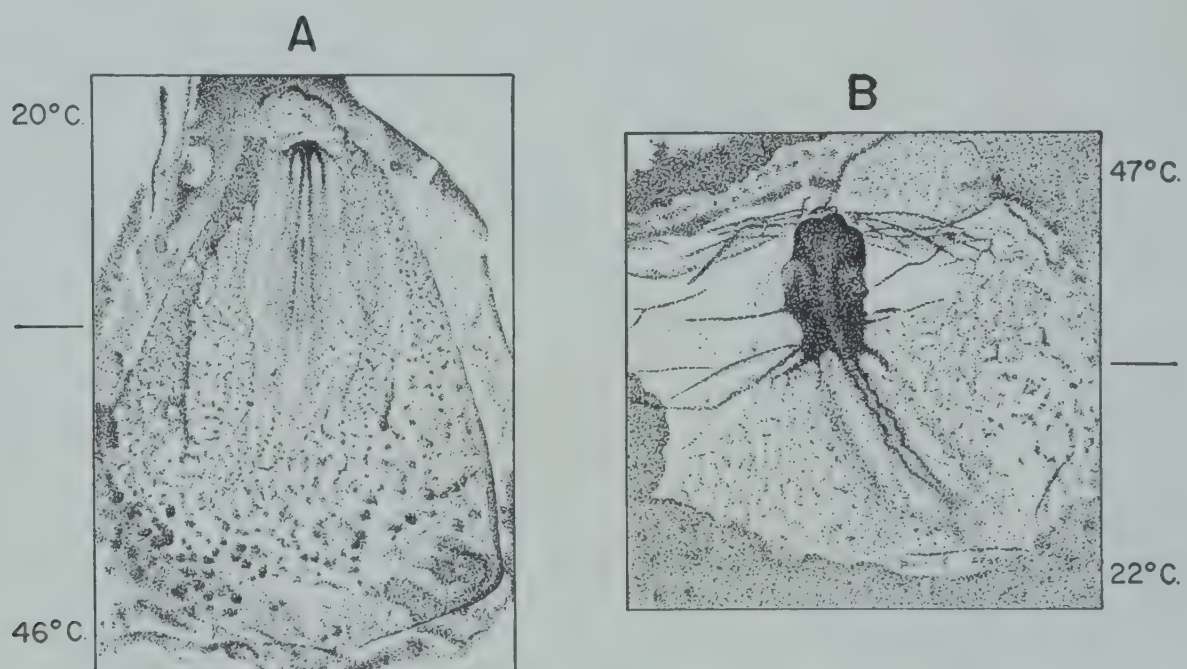
Fig. 79. The relationship between incubation temperature and the formation of somites in the chick embryo. (Drawn from unpublished data of Romanoff.)

Anomalous development detectable within the first day is a frequent result of constant incubating temperatures that are too low. Dareste (1869) remarked that he could produce monstrosities at will by lowering the temperature to 30° to 34° C. He also mentioned the development of blastoderms without embryos at 28° C. (Dareste, 1891, pp. 117, 284). Mitrophanow (1895) described a blastoderm that attained only a 16-hour stage of development after more than 3 days' incubation at 32° to 34° C. Two primitive streaks were joined in a Y-shaped formation, and a third small streak was present in the anterior portion of the area pellucida. Alsop (1919) noted that 50 to 96 per cent of all 24-hour embryos were abnormal when the incubating temperature was not more than 2 or 3 degrees below



the optimum. In many instances observed by this author, the primitive streak was curved instead of straight throughout its posterior one third. Neural structures appeared to be most sensitive to insufficient heat. The neural tube was affected approximately four times as frequently as the brain primordium. Often the neural folds failed to form at one level or another, or a fold developed on only one side. Extra thickenings of the wall of the neural tube were found, especially at the node level, and extra cells were present in the neural canal.

Interruption of the first 24 hours of incubation by a period of cooling may also have a pronounced effect. The embryo may not survive the treatment; if it does, its subsequent development may be abnormal, or normal but delayed.



**Fig. 80.** The effect of a temperature gradient on the early development of the chick embryo and its extraembryonic tissues. (Redrawn after Tazelaar, 1928.)

A, an embryo incubated 74 hours under an anteroposterior temperature gradient of 20° to 46° C.; B, an embryo incubated 72 hours under an anteroposterior temperature gradient of 47° to 22° C. and then for 48 hours at the normal incubation temperature.

As Kaestner (1896) and Kaufman (1934) noted, the chick embryo's ability to withstand cold decreases with increasing age and also depends on the length of the period of interruption and on the temperature during that period. The fact that embryos up to 48 hours of age can survive for 24 hours at 1.25° C. indicates that good resistance to low temperatures characterizes the early incubation period as well as the pre-incubation period (Kaufman, 1934). Grodzinski (1934a) tested the susceptibility of primitive streak blastoderms (incubated 9 to 14 hours) to a temperature of  $-3^{\circ}$  C. and found that only 3 per cent were killed by 30 to 48 hours' exposure to this degree of cold. In 3 days, 15 per cent died, and 5 days of cooling were necessary to kill 80 per cent. Romanoff, Smith, and Sullivan (1938) observed practically no increase in embryonic mortality in eggs taken from the incubator during the first day and kept for 24 hours at 29° C. Gonzales



and Luyet (1951) noted that the anterior portion of the primitive streak blastoderm was capable of resuming growth after being partially dehydrated, frozen in liquid nitrogen, and then thawed in Ringer's solution at 40° C.

In attempting to determine the maximum possible time of exposure to various temperatures at different hours of incubation, Kaestner (1895) made numerous observations. He found that blastoderms were capable of resuming development in eggs that had been removed from the incubator within the first 6 hours and subsequently held 16 days at 21° C. For every additional 12 hours spent in the incubator prior to interruption, survival time at 21° C. decreased by about 3 days and thus was only 7 days for embryos 24 hours old. Much shorter periods of interruption at 5° C. were fatal at all ages: the embryo was able to endure only 10 days of cooling to this point after 6 hours of incubation, or 5 days after 24 hours. Kaestner also remarked that 1 or 2 hours at 5° C. or 10° C. are fatal to the 18-hour embryo unless the egg is held at 21° C. for 2 hours before being cooled further.

Needless to say, development is at a standstill during the interval when the blastoderm is cooled below the minimum temperature required for the evolution of the embryo. When incubation is resumed, embryogenesis at first lags, so that longer than normal periods of time are needed to reach certain stages (*Kaestner, 1895*).

Interruption of incubation leads to the appearance of various types of anomaly if the period of cooling exceeds a certain minimum time, which is less for lower temperatures (*Kaestner, 1896*). Although the embryo may survive exposure to cold, its subsequent development may be profoundly disturbed by a relatively short interval at a low temperature. As Kaestner (1895, 1896) observed, the 6-hour blastoderm may remain alive for 16 days at 21° C., but it cannot withstand more than 7 days at this temperature without evolving abnormally later. For embryos 18 to 24 hours old, a period of 2.5 days at 21° C. is the maximum that permits normal subsequent development, although survival time may be a week at this temperature. Anomalies are caused by a much shorter interruption at 5° C.—42 hours for the 6-hour embryo and 24 hours for the 24-hour embryo. Grodzinski (1934a) has provided data on the teratogenic effect of a lower temperature on the primitive streak blastoderm. Of such blastoderms held 30 to 38 hours at -3° C., only 10 per cent subsequently developed normally, and all the blastoderms that survived 3 days at this temperature were of the anidian type.

Grodzinski (1934a) investigated the manner in which anidian blastoderms are formed after the primitive streak has appeared. The centrifugal movement of cells is the fundamental cause, as it is in younger blastoderms. During refrigeration, the blastoderm shrinks, and it continues to



do so after incubation is resumed, if the cooling period has been sufficiently long. Cooling also causes the primitive streak to become shorter and broader and the primitive groove and folds to disappear. When the egg is rewarmed in the incubator, the primitive streak gradually disintegrates from the posterior end cephalad. Cells of the ectoderm and mesoderm move out centrifugally, and this movement may continue until a hole is formed in the middle of the blastoderm.

### *The Influence of Elevated Incubation Temperatures*

Within the short range of temperatures above  $37.5^{\circ}$  C. which permits development, embryogenesis proceeds at a greatly accelerated rate. Dareste (1891, *p.* 120) observed that the embryo can attain a 2.5- to 3-day stage within 30 hours if the incubation temperature is raised, and Alsop (1919) specified that elevation to  $41^{\circ}$  to  $42^{\circ}$  C. is sufficient to cause evolution to the 36-hour level in 24 hours. The size of the blastoderm at the end of 24 hours provides some index of the effect of increased heat, as the tabulated data reveal (*Romanoff, unpublished*). The regression of the primitive

Incubation Temperature ( $^{\circ}$ C.)	Diameter of 24-Hour Chick Blastoderm (mm.)
37.5	12.0
38.5	12.5
39.5	13.0
40.5	13.5

streak is also hastened (cf. Fig. 78), and the formation of somites is accelerated (cf. Fig. 79). It may be noted, however, that the stimulating effect of increased temperatures is less marked than the retarding influence of decreased heat, degree for degree. Above  $42.5^{\circ}$  C., some deceleration sets in, as is evident in Fig. 79. At  $43.5^{\circ}$  C., somites are formed more slowly than at  $41.5^{\circ}$  C. and, after the first 30 hours of incubation, more slowly than at  $37.5^{\circ}$  C. Warynski and Fol (1884) observed asymmetrical inhibition of development in embryos overheated at 24 hours by a hot thermocautery needle held close to one side of the blastoderm.

Dareste (1869, 1891, *p.* 119) stated that high temperatures lead to exactly the same anomalies as low temperatures and cited (*p.* 284) the anidian blastoderm as an abnormality that is found at either extreme of the temperature range over which development is possible. He also remarked (*p.* 120) that monsters of a more severe type occur at  $43^{\circ}$  C. than at  $40^{\circ}$  C. Alsop (1919) found that the nervous system is affected fully as adversely at  $41^{\circ}$  C. or  $42^{\circ}$  C. as at subnormal temperatures, anomalies being about equally as frequent in the brain region as in the remainder of the neural tube.

Romanoff, Smith, and Sullivan (1938) observed a 28.8 per cent increase in embryonic mortality when eggs were exposed for 24 hours to a temperature of 41° C. at the end of the first incubation day.

### Other External Influences

Exposure of the unincubated chicken egg (either cool or preheated) to X-rays may have a stimulating or an inhibitory effect on early embryonic development, depending upon the X-ray dosage. Small doses, not exceeding 250 r, apparently favor developmental processes, but all doses higher than this are detrimental, if the size of the 24-hour blastoderm be taken as a criterion. The accompanying table (*Bless and Romanoff, 1943*)

X-ray Dose (r units)	Diameter of 24-Hour Chick Blastoderm (mm.)
0	17.0
8	16.6
50	19.0
250	20.8
1000	12.7
2500	9.4
5000	7.3

shows the relationship between the magnitude of the preliminary dose of X-rays and the diameter of the blastoderm at the end of the first day of incubation. The effect of preheating the egg is negligible, doubtless because the blastoderm is not in a completely dormant state before incubation. Measurements of irradiated blastoderms taken at 6-hour intervals during the first incubation day indicate that even the inhibitory dose of 1000 r is stimulating at first and does not exert its retarding effect until well after the twelfth hour.





## CHAPTER FOUR

# *The Nervous System*

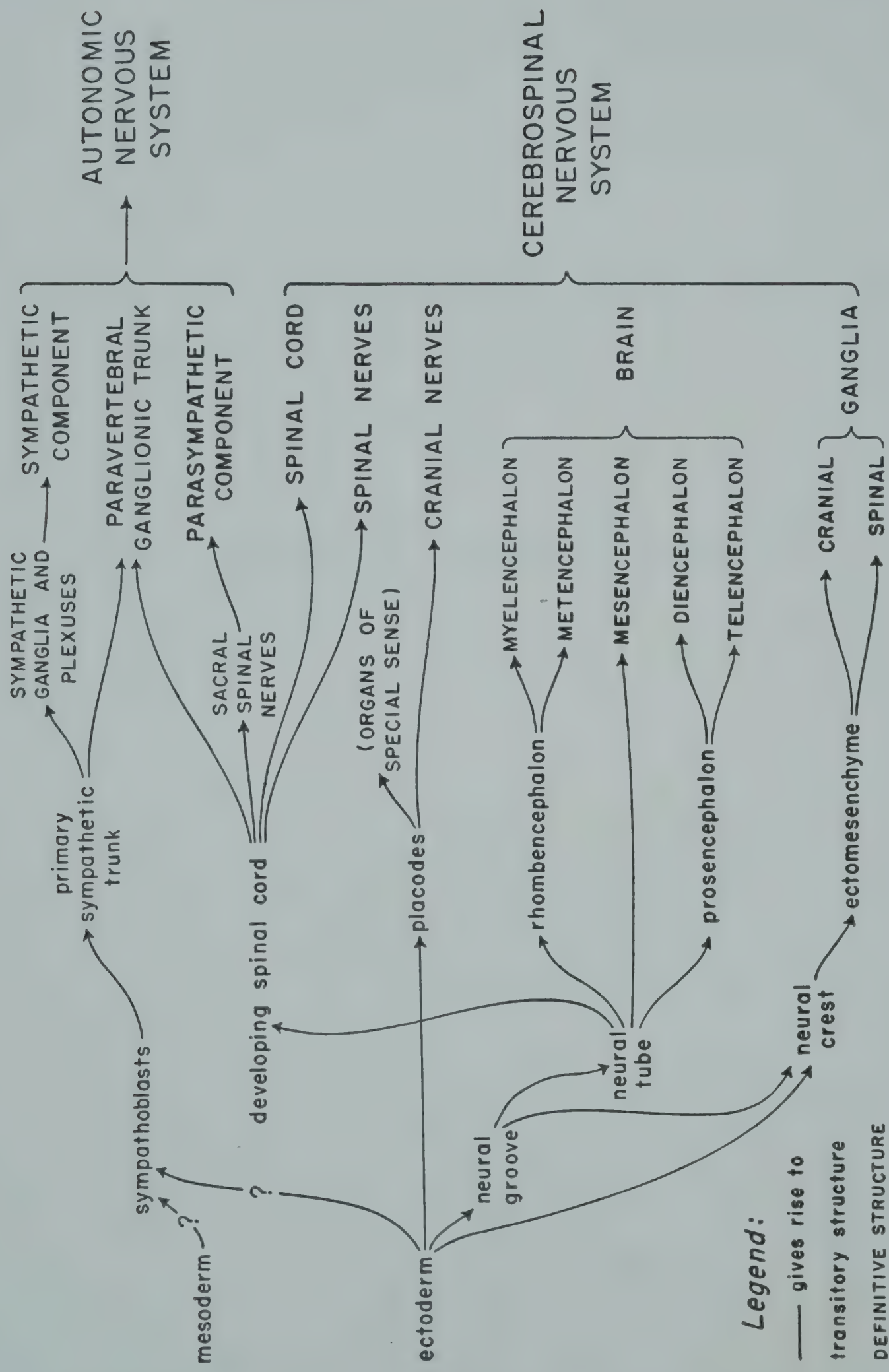
*Its purpose is communication*

*Among the body's parts and cells—*

*To meet each need and situation,*

*At once . . . It has no parallels.*





# THE NERVOUS SYSTEM

The first definite morphological primordium to develop in the avian embryo is that which gives rise to the larger part of the nervous system. The early appearance of primitive neural structure is consistent with the importance of nervous control and coordination in the vertebrate organism.

This very simple neural rudiment is so generalized in structure that it is scarcely distinguishable from the homologous primordium found in many other classes of animal. It undergoes various external and internal transformations and eventually evolves a nervous system in which a characteristic avian configuration is superimposed upon the fundamental vertebrate pattern. In birds, therefore, the nervous system is of the vertebrate type, consisting of an axial aggregation of tissue from which is given off a fibrous network that ramifies peripherally throughout the entire body.

The nervous system is divided into a central and a peripheral portion anatomically as well as functionally. The central nervous system, composed of the mutually continuous brain and spinal cord, contains most of the nerve cell nuclei (cell bodies) and receives, correlates, and initiates impulses. The peripheral network, made up principally of the attenuated cytoplasmic processes of nerve cells, serves chiefly to conduct impulses and so to connect the various parts of the body with the central nervous system. The brain and the spinal cord, and the nerves given off directly from each (the cranial and the spinal nerves), may be considered as forming one division, the cerebrospinal nervous system, as distinct from the semi-independent autonomic nervous system. Many peripheral autonomic nerves are arranged in complicated plexuses containing ganglia, or aggregations of cell bodies, and are only indirectly connected with the central nervous system; but there are also autonomic elements that form an integral part of the cerebrospinal system.

The gross morphological development of the nervous system is inextricably linked with the emergence of the cytoarchitectural features that form the basis of nervous function. The external form of the central nervous system is largely determined by the manner in which the neuronal elements are arranged in functionally similar groups to form definite patterns.

The elements of the nervous system can be classified on a functional basis as either afferent (sensory) or efferent (motor), depending upon



whether they are concerned with impulses that are directed toward or away from nervous centers. There is yet another functional cleavage which extends throughout the entire nervous system and divides it into somatic and visceral elements, the former relating to external adjustments, especially via the skeletal musculature, and the latter to internal adjustments. Visceral elements comprise the autonomic nervous system, but the cerebrospinal system contains additional visceral elements of a specialized type.

## THE FUNDAMENTALS OF NEURAL DEVELOPMENT

Before proceeding further, it is necessary to describe the early scaffolding of the nervous system and to examine the fundamental processes which underlie neural differentiation.

It can be stated almost without qualification that the epiblast furnishes all the neural elements of the body; the origin of only a few is still in doubt. The most important source of nervous tissue is the neural or medullary plate, a rather clearly circumscribed region in the center of the area pellucida where the epiblast becomes thickened at an early stage. Its appearance has been described in Chapter 3. The neural plate is very soon converted into the primitive neural tube, the direct forerunner of the brain and the spinal cord. Aside from the medullary plate, the only other definitely known primordia of neural elements are (1) cells which invaginate from certain small but well-defined areas (placodes) of the surface ectoderm; and (2) neural crest cells, which are found between the neural tube and the surface ectoderm and, being derived from either the one or the other, are definitely of epiblastic descent. In association with cells of neural tube origin, the neural crest and placodal elements enter into the development of the peripheral nerves and ganglia and of the special sense organs.

The epiblast also gives rise to nonnervous elements of the nervous system, for almost all the supporting cells and nerve fiber sheaths are derived from it. The mesoderm, which provides some supporting cells and perhaps certain autonomic nervous elements, is the undisputed source of the meninges surrounding the brain and spinal cord.

The primitive nervous system is composed of undifferentiated cells. At a very early age, these cells begin to multiply and to pass through a series of changes whereby they eventually become nervous and supporting elements. The nervous system, therefore, is evolved through the operation of such basic processes as cellular proliferation, differentiation, and migration. In addition, its development is dependent in large degree upon the apparently directional growth and extension of nerve cell fibers toward particular end-organs, and upon the aggregation of fibers into tracts centrally and into nerves peripherally.



## The Neural Tube

The longitudinal folds along each side of the thickened neural plate close over the neural groove and form the neural tube. The central nervous system develops from this formation and subsequent closure of the neural tube.

### *Formation of the Neural Tube*

In the definitive primitive streak stage the neural plate is oval in shape and occupies a slightly greater area anterior to the primitive pit than posterior to it. During the period of regression of the streak, the neural plate lengthens anterior to the primitive node and flanks the notochord. The neural tube is formed by the fusion of the midline of folds thrown up along the lateral boundaries of the elongated neural plate.

In the chick embryo, the bilateral neural folds first appear shortly after the twentieth hour. They are parallel longitudinal elevations at either side of a groove in the midline; they extend from the level of the head fold to a point somewhat cranial to the anterior end of the primitive streak, where they diverge and fade away (*Mihalkovics, 1877, p. 7*). At this time, the neural folds are neither pronounced nor high, and the depression (the neural or medullary groove) between them is broad and shallow. With considerable rapidity, however, they become more prominent and move closer together, while simultaneously extending themselves posteriorly and (more slowly) anteriorly.

Figure 81 shows a series of cross sections of a 1- or 2-somite chick embryo of about 20 hours' incubation. These sections clearly indicate how the early neural system varies in development from anterior to posterior. The first two sections (Fig. 81-A and B) are taken through the most anterior part of the cephalic region of the primitive embryonic body, A anterior to the anterior intestinal portal and B slightly behind it. In both, the medullary groove is relatively deep. The third section, C, is from a somewhat more posterior level of the cephalic region, where neural development is maximal and the medullary groove is deep and narrow. In the next section, D, taken still farther posteriorly, the neural groove is a wide but well-marked trough. Section E, through the trunk at the somite level, and section F, anterior to the primitive streak, show that the neural groove in these regions is both shallow and narrow. The neural folds are pronounced in the more anterior of the two sections and only beginning to form in the more posterior one, where the medullary plate is broader. At primitive streak levels (Fig. 81-G), the medullary plate extends in a flat plane on either side of the primitive groove, and the neural folds are barely indicated at the lateral borders of the medullary plate.



After the formation of the neural folds, the boundary between the medullary plate and the general surface ectoderm lies at the apex of each fold. The medial slopes of the neural folds, therefore, are composed of prospective neural tube cells and the lateral slopes of prospective epithelial cells.

The neural folds first come into contact with each other in the region of their initial appearance, the region later to become the mesencephalon or second primary brain vesicle. Contact usually occurs at about the 4-somite

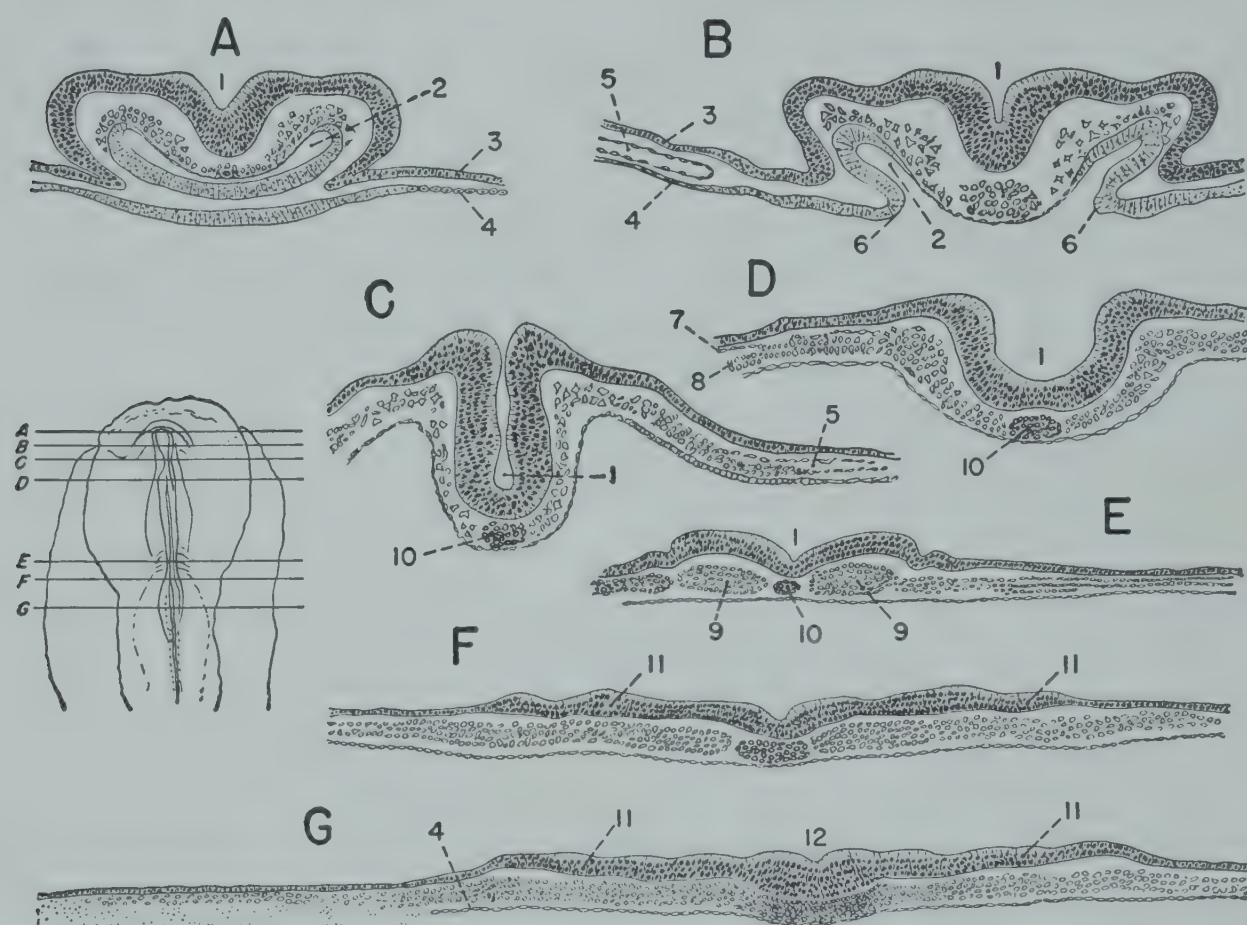


Fig. 81. The formation of the medullary (neural) groove in the chick embryo. (Redrawn with modifications after Duval, 1889.)

A to G, cross sections through a 2-somite (20-hour) chick embryo, showing the variation in the development of the medullary groove from anterior to posterior. All  $\times 68$ .

At left: a dorsal view of the embryo, showing the level of each section ( $\times 6$ ).

1, medullary groove; 2, fore-gut or pharynx; 3, ectoderm; 4, endoderm; 5, peritoneal cavity; 6, edge of anterior intestinal portal; 7, somatopleure; 8, splanchnopleure; 9, somite; 10, notochord; 11, medullary plate; 12, primitive groove.

stage in the chick (Streeter, 1933) and has been observed between the 3- and the 8-somite stages in the European lapwing, *Vanellus vanellus* (O. Schumacher, 1928). The apposition of the folds gives them the gross appearance of having been pressed together with the thumb and forefinger (Streeter, 1933), as Fig. 82-B shows.

At the 5-somite stage (cf. Fig. 82-C), the neural folds form higher ridges and run almost the entire length of the medullary plate. Posteriorly, they diverge around Hensen's node and encompass between them an area of the medullary plate which, because of its shape, is known as the sinus rhom-

boidalis. The neural folds also diverge cranially from the region where they are in contact.

By the time the sixth somite has been added (cf. Fig. 82-D), the neural folds have extended their region of contact farther posteriorly and slightly anteriorly as well. The closed portion of the neural tube is noticeably swollen, the place of greatest fullness marking the location of the future optic evaginations. The 7-somite stage finds the neural folds in close approximation to each other almost as far posteriorly as the primitive pit (cf.

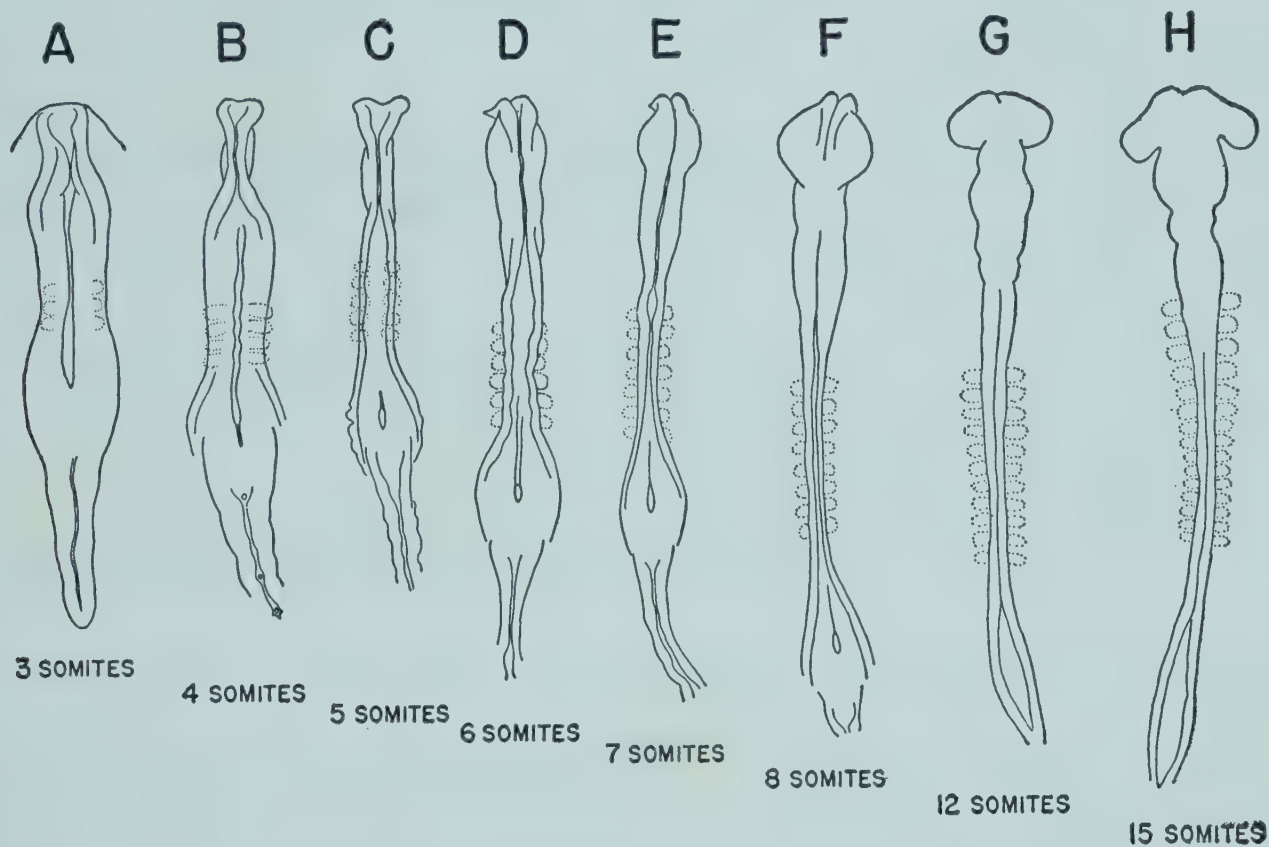


Fig. 82. The formation of the neural tube in the chick embryo. (Redrawn after Streeter, 1933, and Duval, 1889.)

A to H, dorsal views of the primitive nervous system at various stages (the somites are indicated in broken outline). All  $\times 13$ .

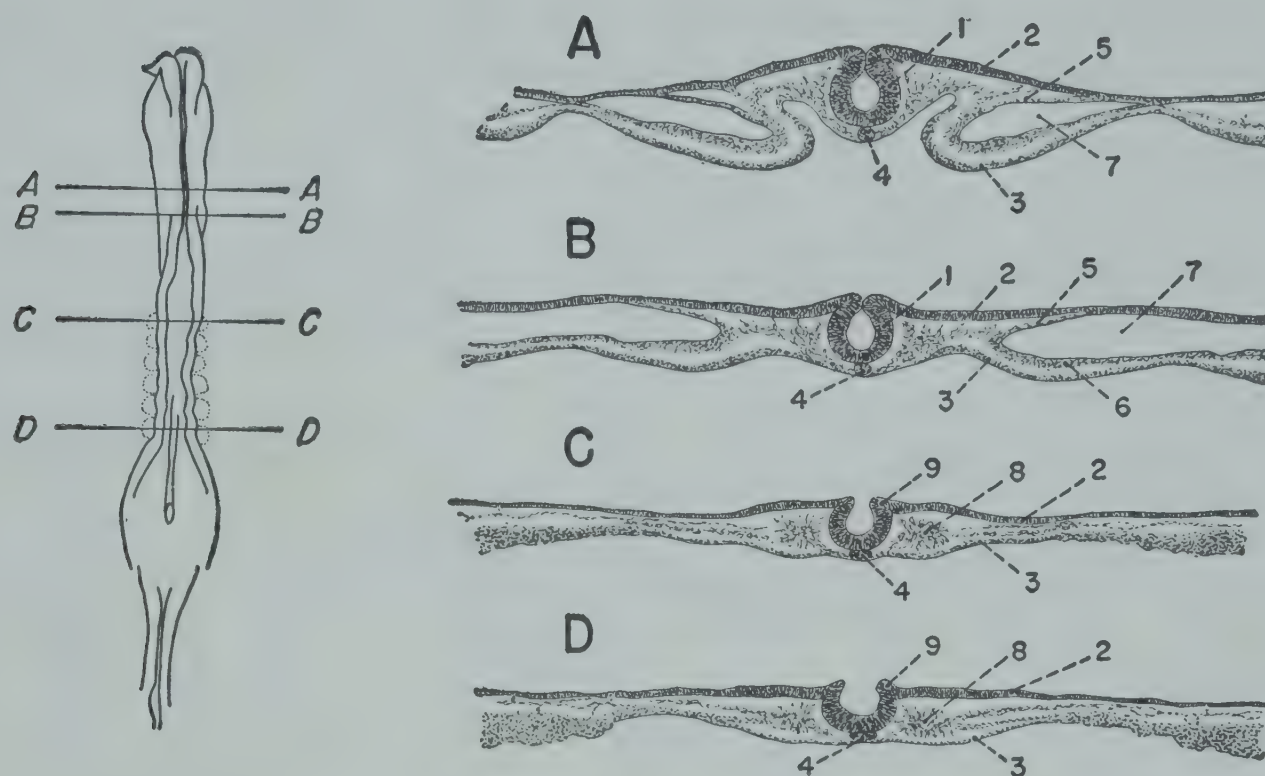
Fig. 82-E). Actual fusion of the folds begins at the 8-somite stage (Streeter, 1933) in the region of first contact (cf. Fig. 82-F); and, at the 11-somite stage (cf. Fig. 82-G), fusion is complete throughout a large portion of the now pronounced anterior swelling, which represents the primordial brain.

The formation of the neural tube is the result of the bending of the neural folds (or, more specifically, of their apical portions) toward the midline until they meet. The medullary plate is thus rolled up into a hollow tube whose roof, consisting of the double folds, is traversed by a longitudinal "seam," or raphe, representing the line of contact between the folds. Cells of the neural crest flank the line of closure, fill the angle between the ectoderm and the neural fold on either side, and intervene between the lips of the neural folds. These neural crest cells migrate laterally and become separated from the overlying ectoderm, which then covers the surface in unbroken continuity. Fusion of the neural folds proper



ensues shortly thereafter. Figure 83 shows four sections from different levels of a single embryo and should clarify the process of neural tube formation.

It is appropriate to insert at this point three diagrams (Fig. 84) presented by Wetzel (1926) to show how the cellular material of the flat neural plate of the definitive primitive streak stage possibly becomes re-



**Fig. 83.** The formation of the neural tube in the chick embryo. (Redrawn after His, 1868.)

A to D, cross sections of the 6-somite chick embryo taken at different anteroposterior levels and showing various stages in the formation of the neural tube by apposition of the neural folds. All  $\times 40$ .

*At left:* a dorsal view of the embryo, showing the level of each section.

1, neural tube; 2, surface ectoderm; 3, endoderm; 4, notochord; 5, somatopleure; 6, splanchopleure; 7, peritoneal cavity; 8, somite; 9, neural folds.

arranged during the period of regression of the streak and formation of the neural tube. The movement of marks placed on the neural plate indicates that the neural material flanking the node becomes greatly elongated in the posterior direction and that the posterior half of the originally oval neural plate is drawn out into the shape of a narrow V, when seen from above. In the closed neural tube, therefore, there is very little level-for-level correspondence with the neural plate, except at the anterior extremity.

A measurable birefringence has been found in the neural plate and tube of early chick embryos. Both form and intrinsic birefringence are present and are due to the submicroscopic arrangement of protein and lipoid components, respectively, of the cells. The birefringence of the initial neural primordia indicates a structural organization that might make possible an imbibition gradient; therefore, the formation of the neural folds and the neural tube may be due to differential imbibition of water (*Hobson, 1941*).



Experimental explantation of early chick blastoderms to various substrates indicates that the formation and closure of the neural folds depend on the presence of utilizable exogenous carbohydrate nutrients, preferably glucose (or mannose). These morphogenetic processes are also very sensitive to oxygen tension and hydrogen-ion concentration; they are supported at pH 7.3 to 9.5 but cannot take place at pH 7.0 to 6.5 (Spratt, 1950b).

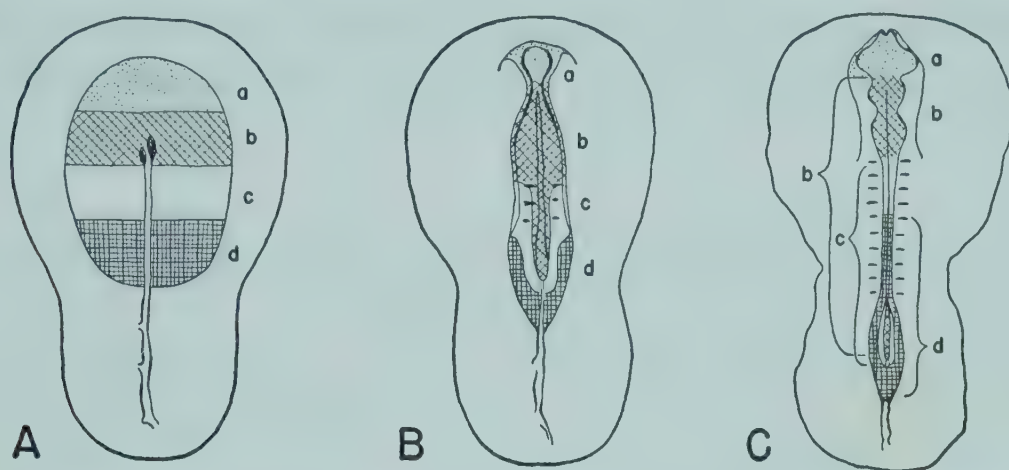


Fig 84. Diagrams to show the possible rearrangement of material of different antero-posterior levels (a, b, c, d) in the chick embryo. (Redrawn after Wetzel, 1926.)

A, the flat medullary plate; B, regression of the primitive streak; C, formation of the neural tube.

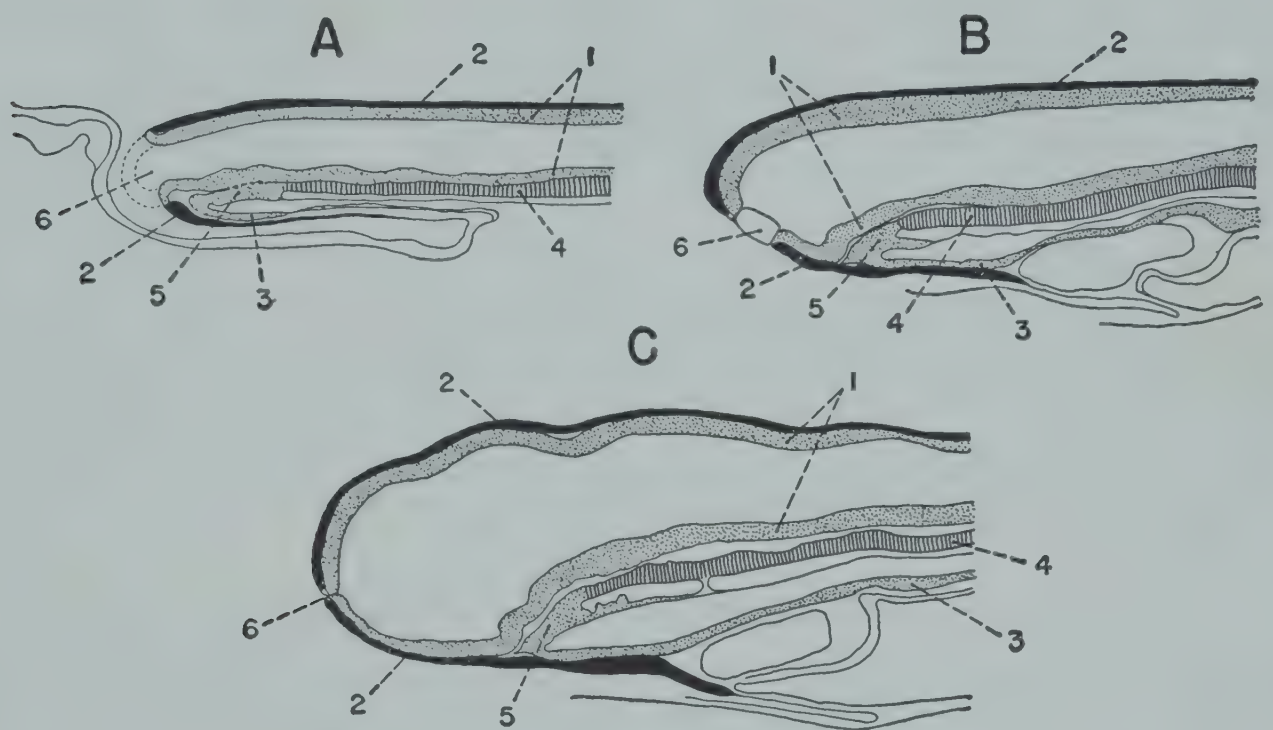
### *Closure of the Neural Tube*

The formation of the hollow neural tube from the originally flat medullary plate continues until both ends of the tube are closed. The anterior end of the tube usually closes in the chick at about the thirtieth hour, during the 12-somite stage (His, 1868, p. 103; Kamon, 1906), although sometimes not until the 16-somite stage (Kingsbury, 1922). O. Schumacher (1928) indicates that closure of the anterior opening (of anterior neuropore) is accomplished in the lapwing (*Vanellus vanellus*) between the 13- and 19-somite stages. In the cranial region, the dorsal portion of the neural folds grows in the longitudinal direction much more rapidly than the ventral portion, or "floor," of the forming brain. Consequently, the folds arch forward and downward, and the originally most anterior point in the medullary plate—the point where the folds are continuous with each other across the midline—is brought to a ventral position (Fig. 85-A and B). This point, theoretically, should represent the anterior neuropore, or point of final closure of the anterior end of the neural tube. Actually, closure proceeds upward and backward from this point at the same time that it proceeds forward and downward along the dorsal line of closure. For this reason, the anatomical anterior neuropore lies dorsal to the theoretical neuropore and is close to the anterior pole of the body (cf. Fig. 85-C). Here the final separation of the anterior end of the neural tube from the surface ectoderm occurs in the chick at the 26- to 28-somite stage (Kupffer,



1906), in the duck (*Anas platyrhynchos*) at 29 somites, and in the European robin (*Erithacus rubecula*) and the lapwing (*Vanellus vanellus*) at some time after the 25-somite stage (Wijhe, 1884)—certainly before the 34-somite stage in the lapwing, *Vanellus vanellus* (O. Schumacher, 1928).

In the chick the closure of the posterior end of the neural tube occurs soon after the formation of the eleventh or twelfth somite (Holmdahl, 1925*b*), during the initial stages of end bud formation (see Chapter 3). Figure 86 shows four sections through the region where the posterior neuropore is being formed by the closure of the neural tube over the sinus



**Fig. 85.** The closure of the anterior neuropore in the chick embryo, shown by sagittal sections. (Redrawn after Kingsbury, 1922.)

A, 6- to 7-somite (26-hour) stage; B, 10-somite (30-hour) stage; C, 16-somite (36-hour) stage. All  $\times 40$ .

1, neural tube (stippled); 2, ectoderm (black); 3, endoderm (stippled); 4, notochord (crossbarred); 5, preaxial mesoderm (stippled); 6, neuropore.

rhomboidalis, which is thus obliterated. In the first section, the almost completely closed neural tube lies above the clearly differentiated chorda; but in the remaining three, the chorda is replaced by the increasingly large mass of undifferentiated cells composing the incipient end bud, and the neural folds are somewhat farther apart. It is plainly seen that the neural groove comes to occupy a position immediately dorsal to the end bud. It appears that the material of the neural plate becomes entirely exhausted in the period between the 11- and the 20-somite stages, and that the task of providing cells for the posterior portion of the neural tube is assumed first by the end bud and then by the tail bud. Apparently the portion of the closed neural tube formed by the last remnant of the neural plate sinks beneath the surface, and the remainder of the neural tube is pro-

liferated out of the end bud as a solid strand which later acquires a lumen (Holmdahl, 1925b; Schumacher, 1927; Seevers, 1932). Several stages in this phase of neural tube development in the 20-somite chick are shown in Fig. 87, which presents five cross sections. In Fig. 87-A, B, and C, it can be seen that the neural tube has two or more lumina. Furthermore, in Fig. 87-C and D, the most ventral cells in the forming neural tube have an undifferentiated character, like the end bud cells in Fig. 87-E, whereas those at the periphery have an orderly arrangement and a definite structure. These facts indicate that the canalization of this portion of the neural tube

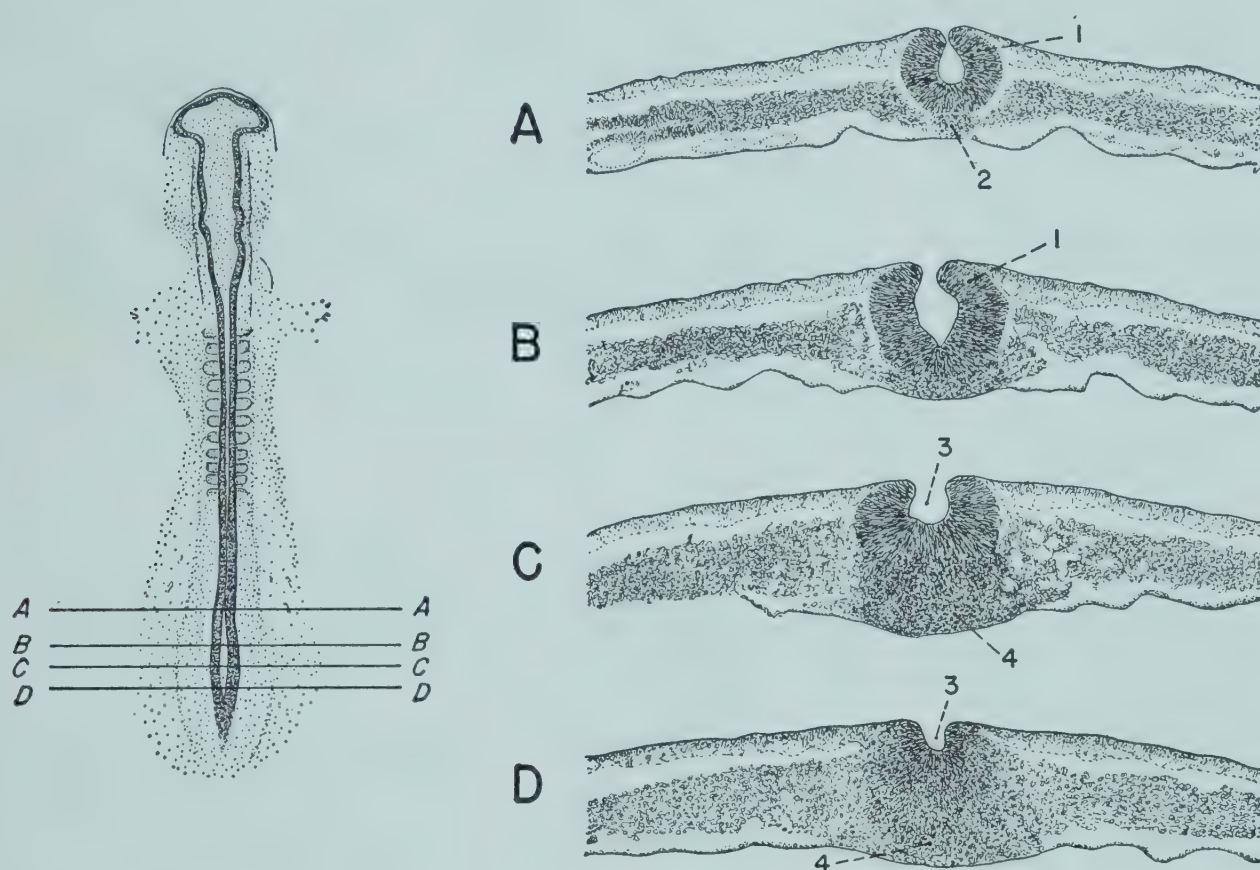


Fig. 86. The relationship of the posterior neuropore to the end bud. (Redrawn after Holmdahl, 1925b.)

A to D, cross sections through the caudal part of an 11-somite chick embryo taken at a time immediately preceding the closure of the posterior neuropore. All  $\times 70$ .

At left: a dorsal view of the embryo, indicating the exact level of each section.

1, neural tube; 2, notochord; 3, neural groove; 4, undifferentiated cells.

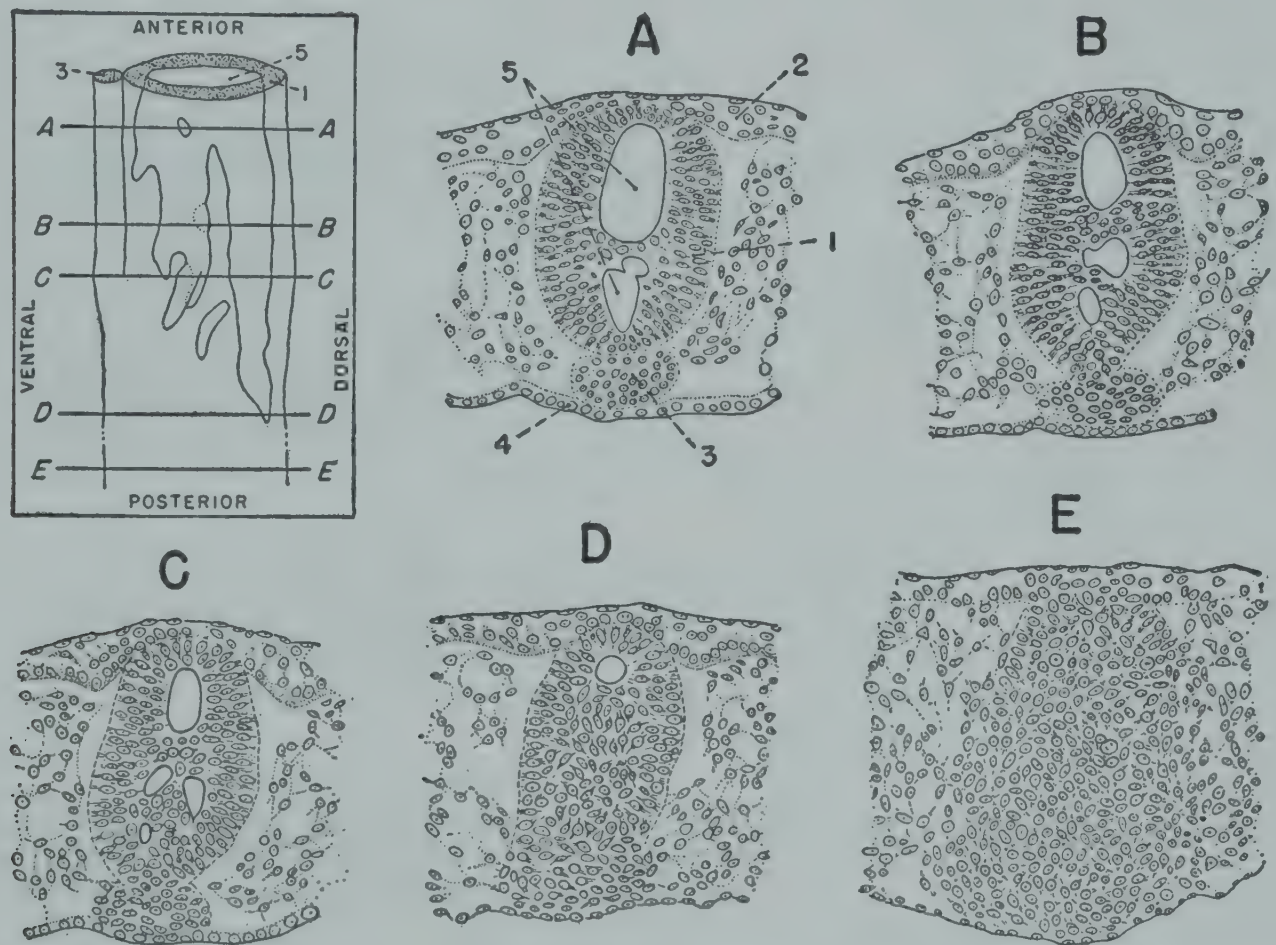
is accomplished by the disappearance of the central cells and the coalescence of several small lumina into one (Holmdahl, 1925b; Schumacher, 1927). According to Holmdahl (1925b), all of the chick embryo's neural tube posterior to the level of the last thoracic segment is formed in this manner.

It is appropriate to mention the fact that multiple lumina in the caudal portion of the chick's neural tube have been observed many times (Gasser, 1879; Mingazzini, 1899; Kelsey, 1911; Fritz, 1914) but have generally been considered anomalous. On the other hand, double or multiple canals in the



midportion of the neural tube (Oellacher, 1875; Kolster, 1899; Smith, 1899; I'ederow, 1907; Gawrilenko, 1924) are probably abnormal, as this part of the tube is too far anterior to be formed from tail bud material (Schumacher, 1927).

The formation and closure of a secondary posterior neuropore has been described by S. Schumacher (1928) in the 12- to 13-day chick embryo. According to this author, a vesicular terminal or preterminal swelling forms in the neural tube because of an increase in internal pressure. The dorsal wall



**Fig. 87.** The formation of a lumen in the caudal portion of the neural tube through the coalescence of several small lumina appearing in a solid structure proliferated from the tail bud. (Redrawn with modifications after Schumacher, 1927.)

A to E, cross sections through the caudal end of the neural tube of a 57-hour chick embryo. All  $\times 150$ .

*Insert:* a diagrammatic lateral view of the same region, showing the level of the sections.

1, neural tube; 2, ectoderm; 3, notochord; 4, endoderm; 5, lumen of neural tube.

of the tube ruptures and the fluid which escapes exerts pressure under the epidermis and produces a second swelling. This also bursts, and the excess fluid flows into the amniotic cavity. The opening soon closes and the defect is covered by epidermis growing from the ventral side. Simultaneously, the caudal end of the neural tube starts to undergo involution and eventually all of the neural tube lying posterior to the end of the vertebral column disappears, although remnants in the form of epithelial vesicles or cell groups persist during embryonic development. In Fig. 88 may be seen the successive stages in the formation and closure of the secondary neuropore, both



in neural tubes which curve around the tail from dorsal to ventral and in those which are straight.

Braun (1880) observed the presence of a cleft in the floor of the neural tube of a 5.5-mm. embryo of the zebra parakeet (*Melopsittacus undulatus*), which corresponds closely in development to the 3-day chick embryo. The cleft, which was 0.2 mm. in length, opened through the ventral wall of

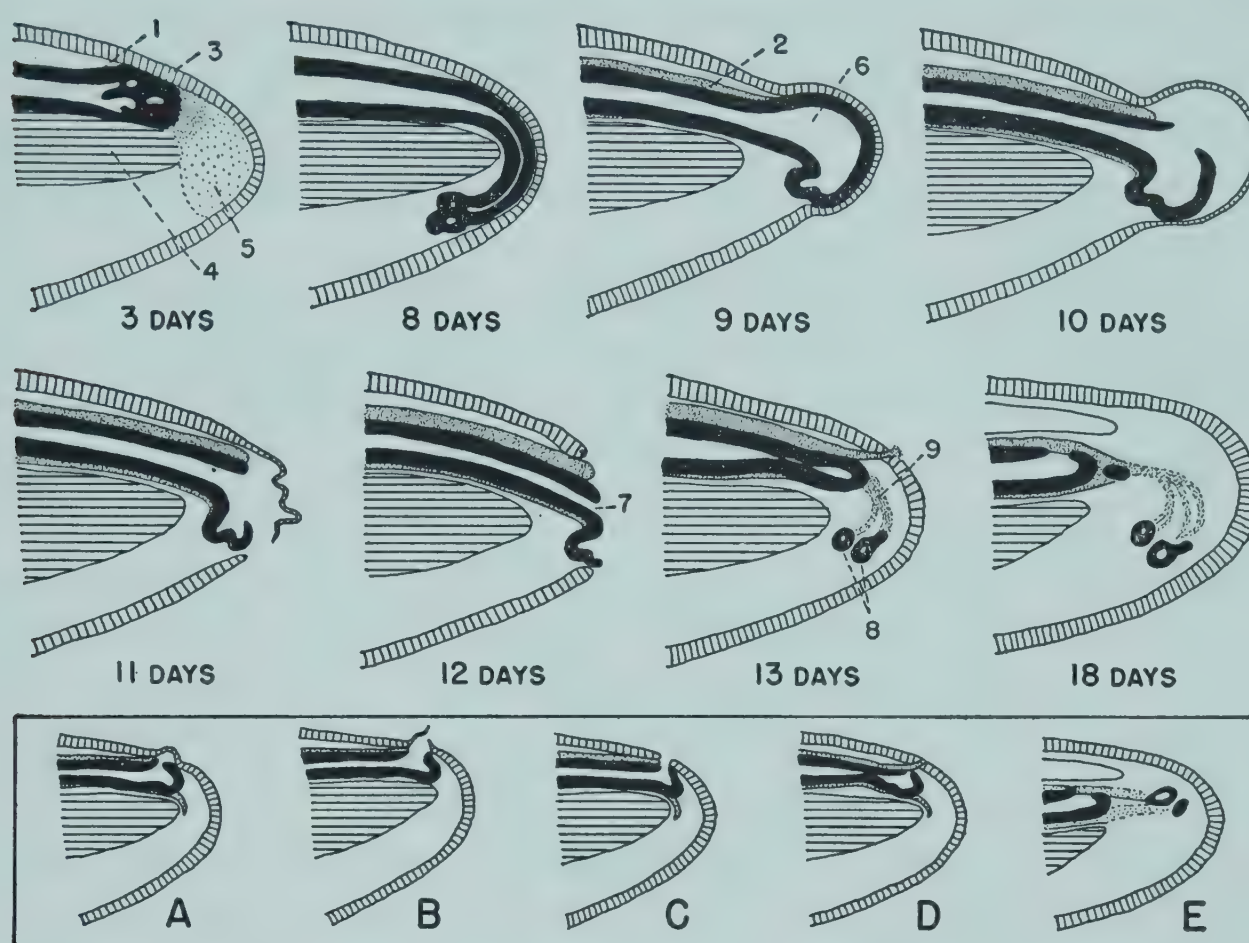


Fig. 88. The secondary posterior neuropore in the chick embryo. (Redrawn with modifications after S. Schumacher, 1928.)

Diagrammatic sagittal sections through the caudal end of the chick embryo's body, to show that the formation and rupture of a terminal vesicle produces a secondary posterior neuropore which eventually closes with accompanying retrogression of the most caudal portion of the neural tube.

*Insert:* diagrams showing the same process as it occurs when the neural tube does not curve ventral to the notochord. A to E, inclusive, represent stages corresponding to those shown in the last five diagrams above.

1, neural tube; 2, peripheral layer of neural tube; 3, surface ectoderm; 4, notochord; 5, tail bud; 6, vesicle; 7, secondary posterior neuropore; 8, remnants of neural tube; 9, unmyelinated nerve fibers.

the neural tube and communicated with the endodermally lined mid-gut. It appeared at the caudal level of the neural tube about 0.1 mm. ahead of the primitive streak. Gasser (1879) and Schwarz (1889) observed a similar occurrence in embryos of the goose (*Anser anser*) and duck (*Anas platyrhynchos*), respectively. Braun (1880), by experimentally retarding the development of the pigeon (*Columba livia*) embryo, observed a rapidly transient communication between the neural canal and the mid-gut.



### The Neural Crest

The neural crest is a distinct but transitory primordial entity. It consists of an aggregation of cells which appears between the ectoderm and the early neural tube. The place where it arises corresponds to the original boundary between the epithelial ectoderm and the neural ectoderm; hence its origin, though clearly ectoblastic, is disputed. Its fate, too, is not yet entirely clear. In the development of the nervous system, it plays a role in the formation of the cranial, spinal, and sympathetic ganglia and of the outer sheath (neurilemma or sheath of Schwann) around the peripheral nerves. There is good evidence that it also makes contributions to other, nonnervous tissues.

The existence of the neural crest in the chick was first noted by His (1868, *pp.* 78, 87), who remarked on the presence of an ectodermal thickening at the juncture of the medullary plate with the ectoderm, at a time prior to the closure of the neural tube. Beard (1888*a*, 1888*b*), Lenhossék (1891), and Goronowitsch (1893) also observed a slight proliferation of cells on either side of the still open neural tube in the re-entering angles between the ectoderm and the erect neural folds. Other investigators (Marshall, 1878; Holmdahl, 1928; Campenhout, 1937*a*) have regarded the neural crest proper as having its inception after the neural folds have made contact with each other but before they have fused.

The neural crest makes its initial appearance at the level where closure of the neural tube begins (the future mesencephalic region). In cross section, the neural crest appears as a small, triangular mass of round cells, several layers deep, flanking the median line of closure on either side (Fig. 89-A and *B*<sub>1</sub>). This mass soon begins to extend laterally (Fig. 89-B<sub>2</sub> and C). The lateral extensions were termed the "neural ridges" by Marshall (1878). Some neural crest cells are now seen to lie in the midline, as if wedged in between the lips of the neural folds (Fig. 89-B<sub>1</sub>). These cells give the neural crest the appearance of issuing out of the middorsal portion of the neural tube (Marshall, 1877, 1878), particularly after the ectoderm fuses in the midline and separates from the underlying cells. The median portion of the neural crest—the portion to which the term "neural crest" was originally applied (Marshall, 1879)—is, however, merely the result of fusion of the two halves in the midline.

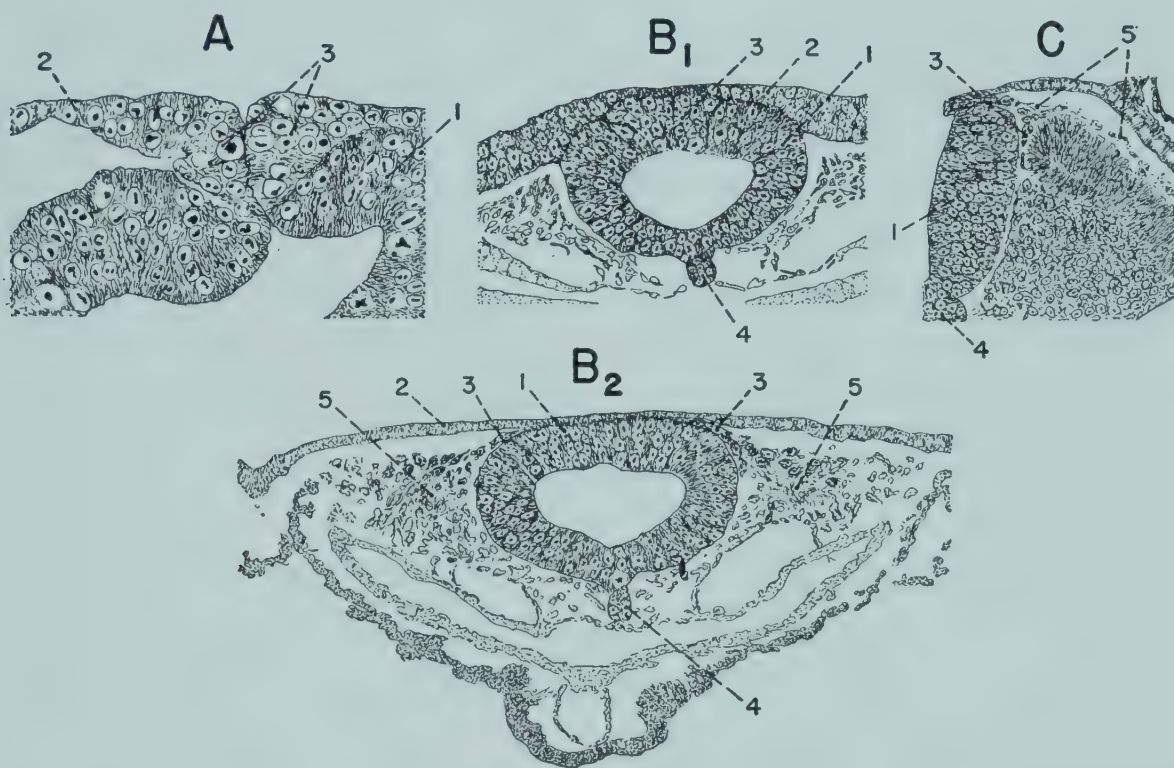
The origin of the neural crest has been the subject of much debate, for opinions have differed as to whether it is derived from superficial ectoderm or from the neural tube. It is quite probable that both these sources contribute to its formation (Holmdahl, 1928; Campenhout, 1937*a*).

Anteriorly the neural crest reaches to the level of the posterior border of the optic vesicles (Holmdahl, 1928; Campenhout, 1937*a*), or possibly as far forward as their anterior border (Marshall, 1878; Goronowitsch, 1893;



*Tello, 1946*). As the process of neural tube closure progresses posteriorly, the neural crest elongates in the same direction. Several stages in the development of the neural crest are shown diagrammatically in Fig. 90.

At the time of its appearance the neural crest is continuous longitudinally, except, possibly, for a short interruption in the anterior part of the rhombencephalon (*Holmdahl, 1928; Tello, 1946*), which is the most posterior of the three primitive brain vesicles or anterior enlargements of the neural tube. The neural crest is considerably more massive in the head region than throughout the trunk, being particularly well developed at the level of the



**Fig. 89.** The neural crest in the chick embryo, shown at several stages in cross sections taken at various levels. (Redrawn with modifications after *Holmdahl, 1928*.)

A, the bilateral neural crest from the region between the forebrain and the midbrain of the 6-somite chick embryo ( $\times 300$ );  $B_1$ , the neural crest after fusion of its two lateral halves in the midline, as seen in a section taken at the mid-rhombencephalic level of the 13-somite chick embryo ( $\times 150$ );  $B_2$  and C, the lateral migration of the neural crest cells, as seen ( $B_2$ ) at the anterior rhombencephalic level in the 13-somite chick embryo and (C) at the upper spinal cord level in the 20-somite chick embryo ( $\times 150$ ).

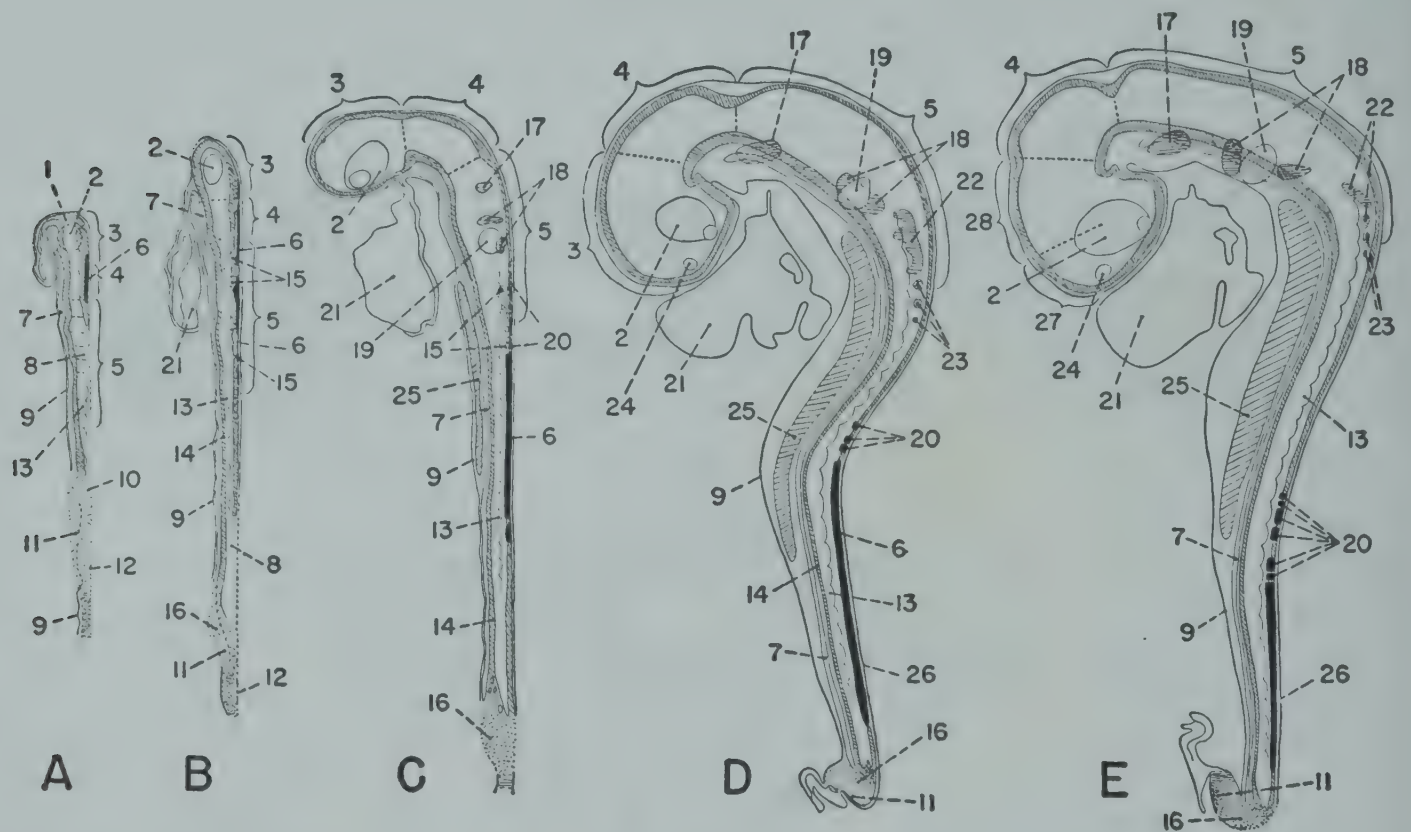
1, neural tube; 2, surface ectoderm; 3, neural crest; 4, notochord; 5, lateral aggregation of cells from neural crest.

prosencephalon (*Campenhout, 1937a*), or first brain vesicle, and in the midportion of the rhombencephalon (*Holmdahl, 1928*). It becomes smaller in the posterior rhombencephalic region (*Goronowitsch, 1893; Campenhout, 1937a*).

At the 8-somite stage, the cells of the neural crest in the cranial region start to migrate laterally, and the neural crest is soon separated once more into a right and a left half as the cells move away from the midline. At the 13-somite stage (cf. Fig. 90-B), there are two especially well-marked accumulations of neural crest cells in the rhombencephalon, one at the anterior



level and the other at the midlevel. The more rostral (anterior) of these cellular aggregations has been traditionally designated as the anlage of the semilunar ganglion (the ganglion of the trigeminal or fifth cranial nerve), and the more posterior as the primordial acoustico-facial ganglion. It is possible, however, that they are predominantly the forerunners of the mesodermal tissue of the first and second branchial arches (*Holmdahl, 1928*).



**Fig. 90.** Successive stages in the development and disappearance of the neural crest in the chick embryo, as shown by schematic midsagittal sections. (Redrawn with modifications after *Holmdahl, 1928*.)

A, 6-somite (3.8-mm.) embryo; B, 15-somite (5-mm.) embryo; C, 20-somite (6-mm.) embryo; D, 23-somite (7.2-mm.) embryo; E, 29-somite (6.5-mm.) embryo. All  $\times 10$ .

1, neuropore; 2, eye; 3, prosencephalon; 4, mesencephalon; 5, rhombencephalon; 6, median neural crest; 7, notochord; 8, neural groove; 9, endoderm; 10, primitive pit; 11, primitive streak; 12, primitive groove; 13, somite; 14, neural tube; 15, lateral portions of neural crest; 16, end bud or tail bud; 17, ganglion of trigeminal nerve; 18, primordial acoustico-facial ganglion complex; 19, otic vesicle; 20, remnants of median neural crest; 21, heart; 22, primordial glossopharyngeal-vagus ganglia; 23, anlage of spinal ganglion; 24, nasal pit; 25, aorta; 26, epithelium; 27, telencephalon; 28, diencephalon.

As the neural crest elements migrate away from the neural tube, they retain their identity for a time, and then they gradually become indistinguishable from the mesenchyme cells with which they mingle. The disappearance of the crest progresses from anterior to posterior. At the level of the brain (cf. Fig. 89-B<sub>2</sub>), where the migrating cells enter a region containing, ventrally, a sparse mesenchyme, the neural crest cells remain in a dorsolateral position, at least for a time (*Goronowitsch, 1893*). At lower levels (cf. Fig. 89-C), they proceed ventrally as slender columns between



the somites and the neural tube (*Onodi, 1884*). Some of them have been observed to migrate outward immediately beneath the ectoderm, dorsal to the somites (*Goronowitsch, 1893; Holmdahl, 1928*).

According to *Holmdahl (1928)*, the crest becomes discontinuous as it disappears, so that remnants of irregular size are briefly present in its most anterior portion, which is found at progressively more posterior levels. In 16- to 20-somite chick embryos (*Fig. 90-C*), the neural crest is no longer distinguishable in the midline anterior to the rhombencephalon (*Campanhout, 1937a; Tello, 1946*). It is irregularly interrupted at several points opposite the first five somites (the first three of which are at a rhombencephalic level). In the anterior part of the rhombencephalon, three large lateral groups or columns of cells (the so-called periaxial cords) represent the vestiges of the neural crest of this region. In 23-somite embryos (*Fig. 90-D*), the metamorphosis of the neural crest has progressed as far as the tenth somite (*Holmdahl, 1928*).

The neural crest may be considered as the source of the ganglion cells and the sheath and supporting cells of the spinal ganglia (*Tello, 1947*), although there is some uncertainty regarding the line of continuity between the neural crest elements and those of the ganglia. Originally, it was thought that the ganglia were derived directly from the neural crest, but this view has been questioned by investigators who regard ganglia, and especially cranial ganglia, as redifferentiations of indifferent or mesenchymatous tissue of neural crest origin. The derivation of part of the head mesenchyme (mesectoderm or ectomesenchyme) from material of the neural crest is no longer disputed. In the cranial region, neural crest cells which do not degenerate or form mesoderm are considered as most probably giving rise to cells of Schwann (neurilemma cells) rather than to nerve cells. It has also been established that certain pigmented cells of the integuments are descended from neural crest elements.

### Histogenesis of the Nervous System

From the undifferentiated ectodermal cells of the neural tube develop all but a very few of the nervous and nonnervous (supporting) elements of the central and peripheral nervous system. There are two major types of cells which differentiate from the primitive cells of the neural plate. These are neurones, the functional cells of the nervous system, and supporting elements (ependymal, neuroglial, sheath, and capsular cells). In addition, a number of peripherally lying neurones and supporting cells arise from the neural crest. From other sources also are derived: (1) the sensory neurones of the olfactory epithelium, the inner ear, and parts of certain cranial ganglia, all of which are contributed by ectodermal placodes; (2) micro- or mesoglia cells (a type of supporting cell), which are usually interpreted as amoeboid phagocytes of mesodermal origin; and (3) the



meninges, or membranes surrounding the central nervous system, which are largely supplied by mesoderm.

The wall of the neural tube is initially composed of a single layer of columnar epithelial cells.<sup>1</sup> Very soon, several layers of cells are present, arranged radially about the central canal. There can now be identified three zones: (1) an innermost, ependymal zone of neural "germinal" epithelium, surrounded by (2) a zone (the mantle zone) composed of undifferentiated cells and occupying the position of the future gray substance, and (3) a peripheral or marginal nonnucleated zone, within which the white substance will develop. An internal limiting membrane lines the central canal, and an external limiting membrane defines the outer surface of the tube. The tissue of the neural tube at this stage is sometimes called a myelospongium.

The "germinal" cells are merely the neural epithelial cells in the active mitotic phase (*Hamburger, 1948*); from them will be derived the neurones and the supporting cells of the brain and spinal cord. The ventricular position of mitoses in the central nervous system is well established, although extraventricular mitoses have been observed (*Milone, 1923*). These may represent dividing mesodermal cells which, having entered via the blood vessels, differentiate into the micro- or mesogial cells.

The daughter cells produced by division of the germinal cells migrate laterally into, and form, the mantle zone. *Hamburger (1948)* suggested that all daughter cells migrate outward and that their places are taken by other neural epithelial cells; he thus explained the skewed curves obtained in mitotic counts. Many authors, however, have held the opinion that only half the daughter cells migrate away from the ependymal layer.

The descendants of the germinal epithelial cells differentiate in two directions. Some become spongioblasts, which either migrate outward to give rise to neuroglial cells, or remain next to the central canal to form cells of the ependymal layer. Others become neuroblasts, the forerunners of the neurones or nervous elements.

It is possible that a function of the embryonic ependymal layer is to secrete the cerebrospinal fluid. Cells of this layer, grown *in vitro*, exhibit the capacity to secrete substances containing proteolytic enzymes, whereas more superficial layers of the brain do not (*Weiss, 1934a*).

### *The Neurone*

The structural and functional unit of the nervous system is the neurone, which is unique among cells in that its cytoplasm is drawn out into attenuated processes superbly adapted for conducting impulses over long dis-

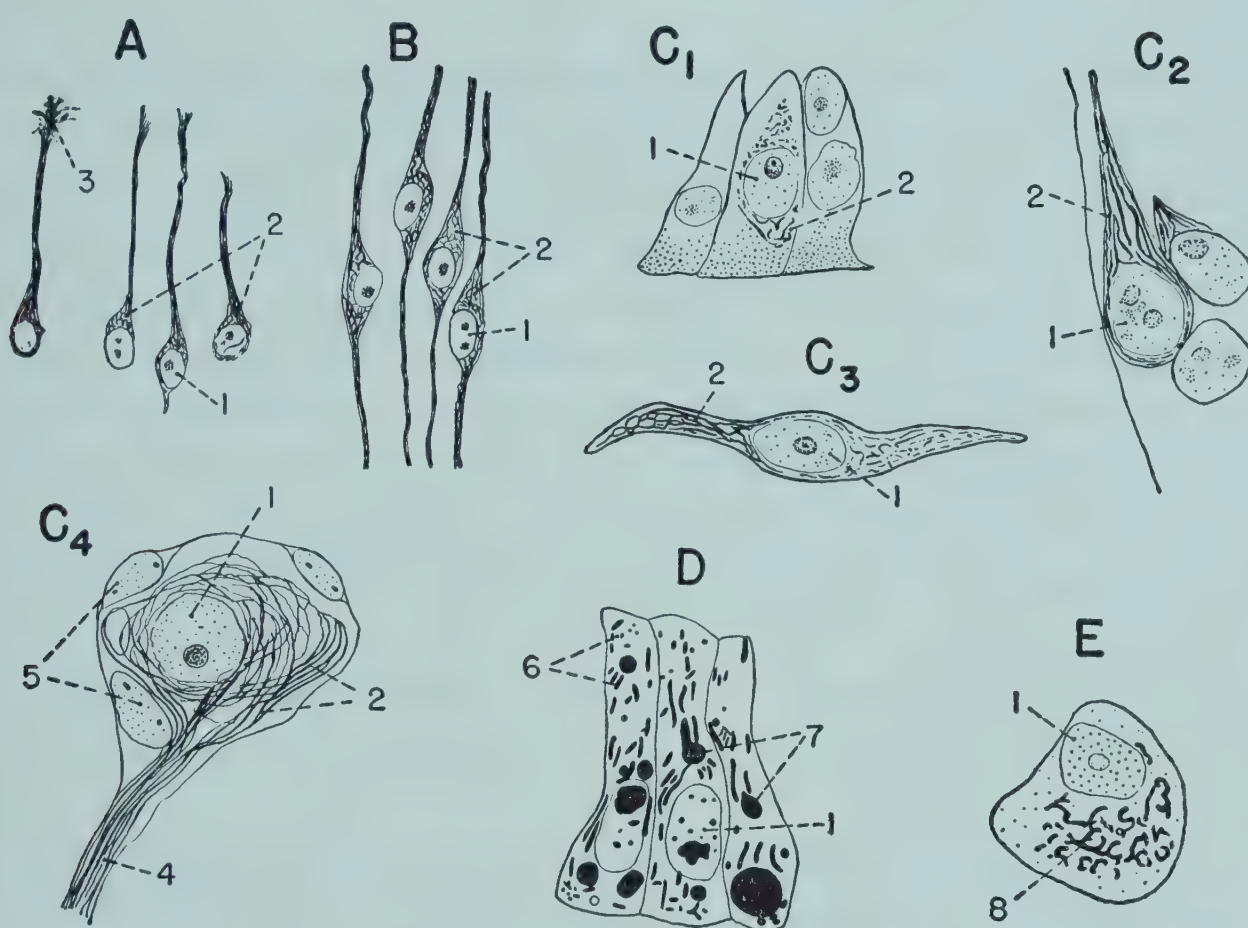
<sup>1</sup> Observations supporting the epithelial as opposed to the syncytial nature of the neural tube have been made by *Duncan (1957)* through an electron microscope study on chick embryos of 24 to 72 hours.



tances. The neurone may be said to be "polarized," for it possesses two types of processes, one which conducts impulses toward the cell body and the other which conducts them away from it.

Neuroblasts, the primordial neurones, are capable of elaborating cytoplasmic processes and of forming functional connections with other nerve cells. Their differentiation into neurones involves the appearance within them of the Nissl material and the neurofibrils characteristic of nerve cells.

The neuroblast, when it arises by mitosis from the germinal neural epithelium of the ependymal layer, is an apolar cell. As differentiation



**Fig. 91.** The differentiation of the processes, neurofibrils, mitochondria, and Golgi apparatus in the neuroblast. (Redrawn with modifications A and B, after Ramon, 1905; C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and D, after Cowdry, 1914; C<sub>4</sub>, after Weiss and Wang, 1936; E, after Cowdry, 1912b.)

A, monopolar neuroblasts from the medulla of a 3-day chick embryo, in which may be seen neurofibrillar structure and, in one, the growing tip of a prospective axon ( $\times 475$ ); B, early bipolar neuroblasts, showing neurofibrillar structure, from a dorsal root ganglion of a 5-day chick embryo ( $\times 475$ ); C<sub>1</sub>, the early stages of neurofibrillar formation in neuroblasts from a 15-somite (40-hour) chick embryo ( $\times 850$ ); C<sub>2</sub>, more completely formed neurofibrils in a neuroblast from the hindbrain of a 40-hour chick embryo ( $\times 850$ ); C<sub>3</sub>, neurofibrils in a bipolar cell from the hindbrain of a 65-hour chick embryo ( $\times 850$ ); C<sub>4</sub>, the course of neurofibrils in the living cell, as seen in a bipolar ganglion cell from an 8-day chick embryo cultivated *in vitro* for 7 days ( $\times 650$ ); D, mitochondria in the undifferentiated epithelial cells of the medullary plate of a 24-hour chick embryo ( $\times 850$ ); E, the Golgi apparatus in a spinal ganglion cell of a pigeon (*Columba livia*), as seen after staining with a 2 per cent osmic acid solution for 8 days ( $\times 425$ ).

1, nucleus; 2, neurofibrils; 3, growing tip of a prospective axon; 4, axon; 5, nucleus of sheath or capsular cell; 6, mitochondria; 7, yolk granules; 8, Golgi apparatus.



proceeds, the cell assumes a pear-shaped appearance, and from its tip there grows out a slender process, the primitive axon, which carries impulses away from the cell body. Figure 91-A shows several monopolar neuroblasts and the enlarged growing tip, or cone of growth, at the distal end of the developing axon. Some time after the formation of the axon, a second process is evulsed from the neuroblast. This is the dendritic process, which makes the cell a bipolar neuroblast (Fig. 91-B) and which conducts impulses toward the cell body. Many cells retain this bipolar appearance; others (such as the spinal sensory ganglion cells) revert to a unipolar or T-shaped appearance; and some develop additional dendritic processes and assume a multipolar condition.

The histological changes in the differentiating neuroblast have occupied the attention of numerous authors. Ramon (1890a, 1905, 1908a) has given some of the clearest descriptions of the process. Reddick (1951) has observed the differentiation of "germinal" neural epithelium cells into monopolar, bipolar, and multipolar neurones *in vitro*, using smears of the postotic medulla of chicks of 2 to 7 days' incubation. That there are two major types of primordial neuroblasts—one capable of self-differentiation (the primary neuroblast), and another which requires some influence exerted by the periphery before it is capable of differentiating (the secondary neuroblast)—was suggested by Shorey (1909) and confirmed by Hamburger and Keefe (1944).

The internal structures of the neurone which have received the most attention are the Nissl material, or chromidial substance, and the neurofibrils.

The Nissl material is now known to be composed, at least in part, of a ribonucleoprotein. It appears as fine granules in the cytoplasm of the cell body (except in the region from which the axon arises) and in the dendrites (Cowdry, 1914). Cameron (1907) regarded it as a product of the nucleus. Hyden (1943) and other workers, employing an ultraviolet light microscope, have indicated that the nucleus does indeed produce this material. Arai (1923) observed the presence of "nucleoproteid-like" particles in the embryonic cells of the chick that were morphologically and histochemically similar to the Nissl substance. He believed that there was a transport of these particles from nonnervous cells into the neuroblasts. The properties of the Nissl material are closely related to the specific functional activity of the neurone and the quantity of this material varies with the activity and the well-being of the cell.

The neurofibrils may be clearly seen in the cytoplasm of the neurone in properly fixed and stained tissue (Fig. 91-C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub>). They have been observed by Collin (1906), Cameron (1907), Ramon (1908a), Gerini (1908), Cowdry (1912a, 1912b, 1914), and many others. However, because no actual fibers were observed in the living nerve cell, the question



of their reality was raised. Weiss and Wang (1936), using dark field illumination, eventually identified a highly refractile material oriented in fibrils parallel to the long axis of the nerve fibers and concentric to the nucleus in the cell body (Fig. 91-C<sub>4</sub>). These workers suggested that neurofibrils may thus be considered as potentially present in the cytoplasm of neurones.

The first neurofibrils have been observed to appear in the neuroblasts of chick embryos at various times—40 hours (Gerini, 1908), 56 to 60 hours (Ramon, 1908a), and 76 hours (Hoven, 1910). Cowdry (1914) reported the first neurofibrils in the neuroblasts of 15-somite, 5.8-mm. chick embryos (Fig. 91-C<sub>2</sub>), which were thus slightly more advanced than Duval's (1889) 33-hour chick.

Ramon (1908a) stated that the first cells to present neurofibrils are apolar neuroblasts in the "germinal" neural epithelium, but Gerini (1908) believed that neurofibrils appear first in bipolar cells in the outer layers of the neural tube. Cowdry (1914) observed neurofibrils in each of these types of cells at stages between 15 and 32 somites.

Hoven (1910) concluded that neurofibrils form in the cytoplasm of the neuroblast. The majority of authors agree that they develop in the immediate vicinity of the nucleus (Cameron, 1907; Ramon, 1908a; Gerini, 1908; Cowdry, 1914) (see Fig. 91-C<sub>1</sub>). Hoven (1910) believed that they form from mitochondria, but Cowdry (1914) indicated that this is not probable.

Nerve cells, in common with other cells, contain Golgi bodies and mitochondria (cf. Fig. 91-D and E).

### *The Growth of Nerve Fibers*

His (1886) stated that nerve fibers are the outgrowths of a single nerve cell, the cell body constituting the generative, nutritive, and functional center of the neurone. This theory, known as the neurone doctrine, has been supported by Kölliker (1905), Ramon (1905, 1908a), Hoven (1910), and numerous other authors. Cameron (1907) stated that the axon arises as a successive extrusion of nuclear-produced material. Lewis and Lewis (1912), demonstrated, by studies of cells *in vitro*, that the nerve fibers of sympathetic ganglion cells arise from a single cell body.

At one time, wide support was also given to the cell-chain theory of Schwann and Balfour (Bethe, 1906; Fragnito, 1908; and others), according to which nerve cells are arranged in long series, the primary cell forming the cell body and the other cells fusing to form the fibers. A modification of this concept, the primitive origin theory of Hensen-Held, proposed that the proximal portion of the fiber is derived from the primary neurone, but that peripherally the fiber enters a syncytium which forms the remainder of the fiber. Most histological observations and experimentation, and what is known of the processes of regeneration, have served, however, to establish the neurone doctrine in preference to the cell-chain theory.



The final neuronal network is the result of an organized and correlated development of tracts and pathways, each composed of many nerve fibers and arising from one or more rather well-defined nuclear or ganglionic masses. It seems probable that this vastly complicated system of connections and interconnections is the result of the coordinated differentiation and growth of individual neurones under the influence of their nervous and nonnervous environment, rather than the product of an indefinite syncytial protoplasmic continuum, as suggested by Hensen (1903) and Held (1909b).

As has been stated, the primordial neuroblast is an apolar cell. The differential development and coordinated growth of an axon from one end of this cell and of a dendrite from the other indicates the influence of some factor or factors. These factors, and those which govern the direction in which a fiber grows once it has appeared, are not entirely clear. Various theories, conceived to account for the apparently well-regulated directionality of nerve fiber development, have placed mechanical, chemical, or electrical influences in the causative role.

His (1887) suggested that mechanical factors, such as the character and arrangement of the surrounding tissues, largely determine the direction taken by a given nerve fiber. His theory emphasized immediate and adjacent forces acting along the pathway to be followed by the fiber, rather than forces operating from a distance. This theory is in sharp contrast with those which propose that the developing fiber exhibits a chemotropic or galvanotropic response to suggested chemical or electrical potential gradients.

The galvanotropic theory promulgated by Strasser (1892), was elaborated by Kappers (1917) into the theory of neurobiotaxis, which has gained wide support. In essence, this theory states that during ontogenetic development, the inherently polarized neuroblast gives rise to nerve processes as the result of electric potential gradients created by stimuli. The axon appears first and exhibits "anode-tropism," growing away from the stimulation center. The cell body and, later, the dendrites exhibit "cathode-tropism" and grow toward the stimulation center.

Bok (1915) restated the galvanotropic theory as the theory of stimulogenous fibrillation. He proposed that the first longitudinal fiber path to form—the medial longitudinal fasciculus—induces the development of axons from the prospective motor neuroblasts as it grows past them. These axons then grow out at right angles to the course of the longitudinal fibers. The induction of the development of axons at right angles of coursing fiber systems continues to underlie the inception and directionality of fiber growth.

Chemotropic theories have been proposed, notably by Ramon (1908b) and Forssman (1900). Such theories imply that nerve fibers grow toward,



or away from, the source of some specific chemical agent. Such a mechanism requires a constant and stable chemical concentration gradient and a selective sensitivity of the nerve fiber for the particular agent (Weiss, 1941).

Both the galvanotropic and chemotropic theories have been based upon speculation, without the support of adequate experimental data. In fact, numerous workers have presented data which refute these theories. Several papers have been published (Weiss, 1934*b*; Karssen and Sager, 1934; Williams, 1936; Gray, 1939) which show that electrical fields have no effect on outgrowing nerve fibers. Rhines and Windle (1944) observed that the cranial motor nuclei in the chick develop normally even in the absence of all longitudinal tracts. Thus it seems that what axial electrical gradient exists in the cell has no demonstrable effect upon the directionality of fiber growth. On the other hand, Ingvar (1920) observed that passage of direct currents (of "density" of 0.001 to 0.002 microamperes per square millimeter of cross section of conducting medium) caused fibers to grow out from explants of chick embryo neural tissue along the lines of galvanic force, that is, toward the anode and the cathode. Marsh and Beams (1946) countered with the observation that direct currents must be of at least 120 microamperes per square millimeter density to influence the outgrowth of nerve processes, which are suppressed on the anode side of explants and elsewhere deflected toward the cathode. It should be noted, however, that this threshold current density represents an electrical potential gradient of 64.2 microvolts per micron and that potential gradients of this order are not unattainable physiologically.

As for the chemotropic theory, Weiss (1934*b*) failed to observe any direct attraction of nerve fibers toward sources of chemical emanation *in vitro*; and the report of reciprocal fiber growth is not compatible with any chemotropic theory. These findings, coupled with the fact that the basic requirements of a suitable chemical gradient are not, and could not, be met in nature, deny the importance of extraneous chemical agents in directing the course of development of nerve fibers.

We are left with the third group of theories, the mechanical, which more recent authors (Weiss, 1934*b*, 1941) have attempted to reinterpret. It is very probable that factors other than the activity of the neurones themselves take part in the determination of the final composite nerve pattern; that is, there are external, nonnervous forces in action. The course of a nerve fiber may be devious from the beginning, or it may become so by distortion from a comparatively straight path. Developing nuclei may actively migrate, as suggested by Kappers (1917), or may be passively displaced by the advent or growth of adjacent structures. The nervous system develops serially, and each structure is influenced by nuclei and pathways already present and by those which develop subsequently.



Weiss (1941) has proposed that the establishment of nerves or pathways entails three overlapping phases: (1) the *free* outgrowth of "pioneering fibers," whose destination, in the embryo, may be actually only a millimeter or so distant; (2) the *bound* outgrowth of additional fibers along the course of the "pioneering fiber," by which the full complement of axons in the nerve or pathway is established; (3) the *towing* process, by which the nerve or pathway is elongated through the growth and displacement of the terminus of the fibers.

The growth of "pioneering fibers" is random, but the pseudopodia at their tips are compelled to follow phase boundaries. These boundaries need not be macro- or microscopic, and are, in fact, most often ultramicroscopic in character. Stereotropism, the colloidal-mechanical phenomenon of attraction, explains the attraction existing between the fiber and the phase boundary, but the often unrelated directionality of nerve fiber growth indicates a selective rather than a common adhesivity. Weiss (1941) further proposed that once a pioneering fiber reaches its terminus some change occurs in its surface, and fibers which grow out later selectively follow its course. This phenomenon he called "selective fasciculation."

The thesis that a given fiber has a single functional potentiality, that it is predetermined to innervate one, and only one, organ is untenable. The primordium of the organ to be innervated undoubtedly plays a part in establishing the orientation of the phase boundaries between it and the axis of the embryonic body (and the location of its source of innervation); but many successful cross connections between nerves and strange terminal areas have been made experimentally, and the invariable predestination of a certain nerve for a particular end-organ cannot be accepted.

Thus it must be concluded that the growth and development of nerve fibers are dependent upon a complex interrelationship existing between the inherent capabilities of the neuroblast and its immediate environment. Mechanical forces, mediated by biophysical principles not yet well understood, result from the total genetic and biochemical environment of the embryo and must be considered, for the present, the principal factors governing the direction of nerve fiber growth in ontogeny.

*The effect of certain drugs on the growth of nerve fibers.* Painter, Pomerat, and Ezell (1949) have tested the toxicity of some twenty-three substances on the growth of nerve fibers *in vitro*. The nerve material consisted of explants from the spinal cord of 9-day chick embryos. The drugs included strychnine, caffeine, atropine, picrotoxin, lobeline, coramine, metrazol, benzedrine, ephedrine, nicotine, camphor tetrazole, cocaine, morphine, barbiturates, sodium bromide, chloral hydrate, and paraldehyde. Drugs or other chemicals normally present in the body included epinephrine, insulin, nicotinamide (niacinamide), biotin, folic acid, glutamic acid, and para-aminobenzoic acid. All but biotin, folic acid, and para-aminobenzoic



acid caused some inhibition of nerve fiber growth. The molal concentrations causing complete inhibition of growth are shown in Fig. 92.

Hudspeth, Swann, and Pomerat (1950) have observed that oxygen concentration has an effect on the proliferation of nerve cells *in vitro*. In their cultures of spinal cord cells from the 9-day chick embryo, fiber outgrowth was increased in high concentrations of oxygen ( $O_2$ ) whereas low concentrations of oxygen (simulating the ischemic condition) produced an outgrowth of glial cells. They speculated on the effects of ischemia in gliosis and the scarring of nerve tissue which often hinders or prevents nerve regeneration.

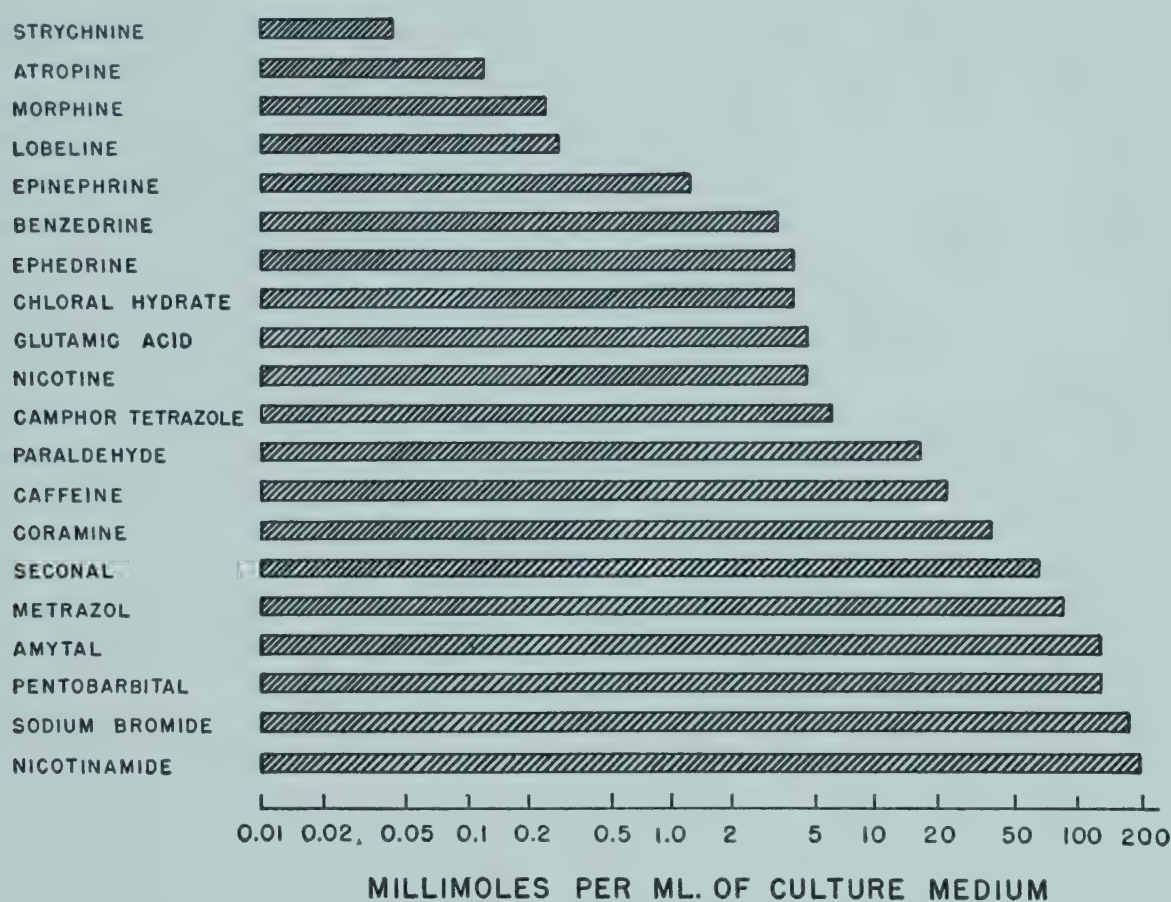


Fig. 92. The varying concentrations of different chemical substances required for total inhibition of fiber outgrowth from chick embryo spinal cord explants *in vitro*. (Redrawn with modifications after Painter, Pomerat, and Ezell, 1949.)

## THE CEREBROSPINAL NERVOUS SYSTEM

The cerebrospinal nervous system consists of the central nervous system (the brain and the spinal cord) and those peripheral fibers whose cell bodies lie in the central nervous system or in the cranial or spinal ganglia. The cranial and spinal nerve roots place the central and peripheral subdivisions in mutual continuity. Within the cerebrospinal nervous system there are also elements that are part of the semi-independent autonomic nervous system.

In contrast to the peripheral nervous system, which merely conducts impulses, the central nervous system contains primary nerve centers that receive and send out impulses and secondary centers that effect adjustment, correlation, association, regulation, and direction. It also contains fibrous



conduction systems, through which centers at various levels of the brain and spinal cord are placed in communication with one another and with the periphery.

There is a genetic and functional unity extending throughout the central nervous system. The brain and the spinal cord are both derived *in situ* from the neural tube, of which the brain is an enlargement at the anterior end, where the walls become irregularly thickened. Although the cytoarchitecture of the brain is more complex than that of the spinal cord, the two structures are organized in fundamentally the same way. As His (1888) stated, the central nervous system of all vertebrates is composed of longitudinal zones, or plates, of functionally similar elements. The zones of the spinal cord are continuous with those of the brain stem.

These zones, four in number, are the roof, alar, basal, and floor plates. The roof and floor plates are located dorsal and ventral, respectively, to the central canal and are composed primarily of nonnervous tissue. The roof plate represents the dorsal seam of the neural tube, and the floor plate is formed of material laid down by Hensen's node as it regresses. The lateral walls, on each side, consist of the alar and basal plates. The former is dorsal to the latter and is separated from it by a groove, the sulcus limitans, which appears early in embryonic development. Only the roof and alar plates extend to the anterior end of the brain; the basal and floor plates terminate at different lower levels, for reasons inherent in the mode of origin of the neural tube from the flat medullary plate.

The alar plate contains sensory components constituting the dorsal or dorsolateral gray (sensory) column in the spinal cord and sensory centers in the brain. The basal plate contains motor components and comprises the ventral or ventrolateral gray (motor) column in the cord and motor centers in the brain. There is a further division of each plate according to the type of end-organ that is innervated—somatic or visceral. The visceral zones on each side of the spinal cord are adjacent to the sulcus limitans and intervene between the somatic zones. With the transition from spinal cord to brain, there is a flattening of the neural tube which shifts the sensory components laterally and leaves the motor components in a medial position. In addition, a special visceral motor column can be distinguished at this level, medial to the general visceral motor components; it supplies muscles of branchial arch origin. Also, lateral to the general somatic sensory column, there is a special somatic sensory column associated with the sensory apparatus derived from the otic vesicle. General visceral motor and sensory elements comprise the cerebrospinal division of the autonomic nervous system.

The peripheral nervous system arises at both cranial and spinal levels by means of paired nerve roots whose elements are in direct connection with the various columns of the central nervous system. The spinal nerves, which



are segmentally arranged in all vertebrates, are of fairly homogeneous composition and therefore easily analyzed. In birds—the highest vertebrates next to mammals—the twelve pairs of cranial nerves no longer possess any segmental character nor exhibit any regularity in their spatial relationships or functional composition. For this reason, the cranial nerves must be considered individually.

### The Brain

The anterior end of the primitive neural tube, dilated almost from the beginning, gives rise to the brain. Through the constriction and the non-uniform thickening of the walls of this part of the neural tube, the various morphological and functional divisions of the brain are differentiated.

In birds, the principal divisions are as follows, from anterior to posterior: (1) the forebrain, or telencephalon, divided into the two cerebral hemispheres; (2) the thalamus, or diencephalon; (3) the midbrain, or mesencephalon; (4) the cerebellum, or metencephalon; and (5) the medulla oblongata, or myelencephalon. The cerebral hemispheres and the cerebellum are phylogenetic developments not found in primitive vertebrates and are sometimes termed the suprasegmental apparatus, in distinction to the remaining or “segmental” part of the brain, known as the brain stem.

As the brain develops, the lumen of the neural tube becomes transformed into the narrow brain cavities or ventricles. The first two, the lateral ventricles, are found within the hemispheres; the thalamus encloses the third ventricle; and, at the midbrain level, a canal (the aqueduct of Sylvius) leads through a constriction, the isthmus, to connect the third ventricle with the fourth, which is the lumen of the medulla. The fourth ventricle is continuous with the central canal of the spinal cord.

The nervous systems of the various classes of vertebrates are distinguished chiefly by differences in the brain. The bird's brain, which bears a closer resemblance to the reptilian type than to any other, is broader and shorter than that of lower forms (see Fig. 100). The cerebral hemispheres are relatively large, pointed anteriorly, broad posteriorly, and unconvoluted. They are characterized by the poor development of the olfactory lobes and the cortex, and by the excellent development of the corpora striata, which comprise most of the substance of the hemispheres. The thalamus is hidden from view. The midbrain is remarkable because of the great size of the optic lobes, which are found in a lateral position on either side of the median, transversely foliated cerebellum.

### Gross Morphological Development of the Brain

The gross development of the brain can be divided into two phases. The first is the establishment of relatively simple structures and their evolution into the various morphological divisions of the brain. In the chick,



this purely formative phase begins with the differentiation of the medullary plate and extends through the fourth or fifth day of incubation (*Kuhlenbeck, 1936*). The second phase, starting on the fifth or sixth day, embraces structural modifications resulting from such processes as the differentiation and proliferation of nervous elements within the brain substance and the establishment of centers and tracts; in short, from the emergence of definitive cytoarchitecture. In the chick embryo, the essential features of the adult avian brain are present by the twelfth day. A third, transitory stage in development has also been recognized (*Kuhlenbeck, 1929, 1936, 1937*), intermediate to and overlapping the others. During this period (extending from the fourth to the ninth day, in the chick embryo), the brain is divided into longitudinal zones which are found at some time in the brain of every vertebrate but which bear no relationship to the functional longitudinal zones.

### *The Early Phase of Development*

The early phase of brain development is characterized by alteration in size, shape, and axial direction of the anterior portion of the straight neural tube. As the result of localized proliferation and growth, as well as of bending and folding, a structure of considerable complexity is formed.

It has been customary to think of the embryonic brain, in its formative period, as being composed of three primary vesicles which are soon subdivided into secondary ones. The brain-vesicle concept is an aid in regional localization (*Streeter, 1933*); it does not imply an equivalence between the various vesicles (*Kuhlenbeck, 1935*), whose orderly appearance denotes the early determination of definite and identifiable brain parts (*Streeter, 1933*).

As previously stated, the development of the brain may be considered to begin with the differentiation of the medullary plate. It will be sufficient, however, to return only to the period when the neural tube is starting to close, for it is at this time that the first recognizable primordia of brain structures become apparent.

*From the 3- to the 12-somite stage.* It has already been noted that the forming neural tube of the 3- or 4-somite chick shows an anterior bilateral fullness presaging the evagination of the optic vesicles. By the time the 6- to 7-somite stage is attained, these swellings are pronounced, and from now on their identity is unmistakable (cf. Fig. 82). Although the neural tube is not yet closed on its dorsal surface, and no true vesicle exists, the eye primordia have been regarded as constituting the forebrain vesicle (prosencephalon) at this time. Figure 94-A is a cross section through the optic evaginations of a 6- or 7-somite chick embryo.

During this same period (the 6-somite stage), the neural tube also becomes enlarged in the region lying directly behind the optic evaginations



(cf. Fig. 82-D and E). The newly expanded portion tapers off posteriorly and presents the appearance of an elongated cone, or spindle.

At the 8-somite stage (cf. Fig. 82-F), a weak constriction (the early sulcus rhombomesencephalicus) appears in the posterior conical part of the brain primordium. This constriction marks the boundary between the hindbrain (or rhombencephalon), lying posteriorly, and the midbrain (or mesencephalon), which intervenes between the hindbrain and the forebrain. The three so-called primary brain vesicles are thus established. The mesencephalon is the primordium of the bilateral optic lobes and the remnant of its lumen will become the aqueduct of Sylvius. The rhombencephalon is yet to be divided secondarily into the metencephalon (where later the cerebellum and pons will differentiate) and the myelencephalon (the forerunner of the medulla oblongata). The lumen of the rhombencephalon will become the fourth ventricle.

At the 11- to 12-somite stage (cf. Fig. 82-G), another weak constriction (the early sulcus mesodiencephalicus) appears anterior to the mesencephalon. The portion of the brain tube lying between this constriction and the optic vesicles is the diencephalon, and the cavity in this region will form the third ventricle. An indentation behind the optic vesicles marks the anterior boundary of the diencephalon. The diencephalon is the first of the "secondary" brain vesicles to appear and is usually considered to be a derivative of the prosencephalon (*Kamon, 1906; Kupffer, 1906; Meek, 1907; Rendahl, 1924; O. Schumacher, 1928; Kuhlenbeck, 1935*).

Also at the 11-somite stage, the forebrain shows a slight enlargement anterior to the optic vesicles. This enlargement is the first indication of the development of the telencephalon, the region where the cerebral hemispheres will form. In addition, the forebrain has begun to turn downward, initiating the bending process which will soon produce the cranial flexure and later almost double the brain upon itself. This change may be seen in Fig. 93-A, which gives a lateral view of the brain of an 11-somite chick embryo in a slightly more advanced stage of development than that shown in Fig. 82-G. The optic vesicles have evaginated further (cf. Fig. 94-B), and the ganglion of the trigeminal (V cranial) nerve has made its appearance on the lateral wall of the hindbrain.

As a sagittal section (Fig. 93-A<sub>2</sub>) shows, all enlargements of the brain tube at this stage represent dilatations of the lumen rather than thickenings of the wall. The dilated regions are separated by internal ridges which correspond to the external sulci. The three most anterior dilatations each form a brain vesicle; but the rhombencephalon consists of several serially arranged lateral pockets or bulges similar (except for their smaller size) to those lying more anteriorly. The presence of these "segments" was noted by such early workers as Baer (1828*b*), Remak (1855), and Dursy (1869). The subdivisions of the hindbrain are not constant in appearance



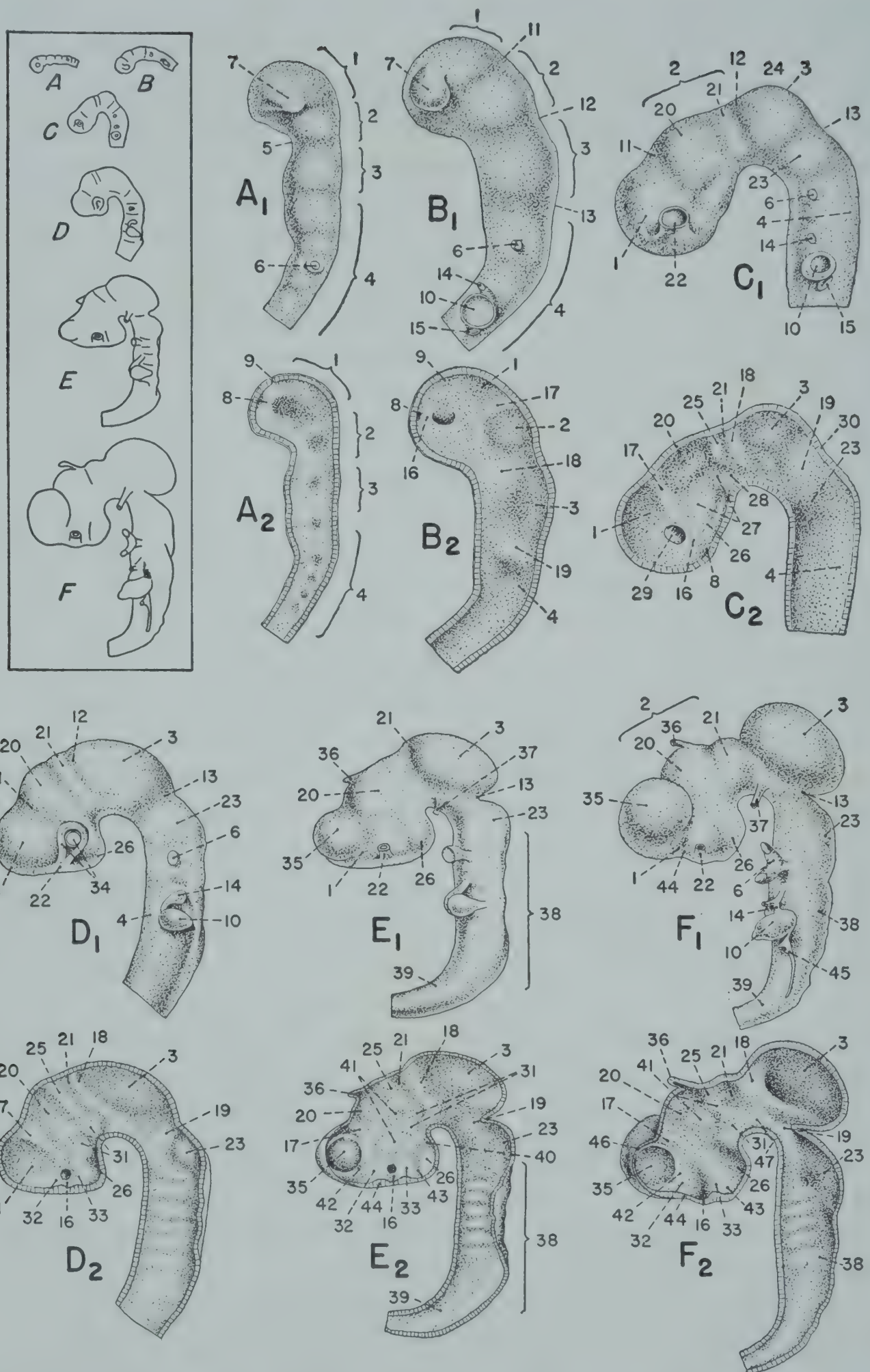


Fig. 93. The development of the chick embryo's brain from the 11-somite to the 50-somite stage, inclusive, as shown by external lateral views and by internal views visible after midsagittal section. (Redrawn with modifications after Kamon, 1906.)

$A_1$ ,  $A_2$ , at 11 somites;  $B_1$ ,  $B_2$ , at 15 somites;  $C_1$ ,  $C_2$ , at 20 somites;  $D_1$ ,  $D_2$ , at 36 somites;  $E_1$ ,  $E_2$ , at 40 somites;  $F_1$ ,  $F_2$ , at 50 somites.

*Insert:* drawings indicating the relative size of the brain at the same stages, shown in A to F, inclusive. All  $\times 4.5$ .

1, prosencephalon; 2, diencephalon; 3, mesencephalon; 4, rhombencephalon; 5, plica



or number, although five or six are usually apparent; as many as eight (*Zimmermann*, 1891) and thirteen (*Meek*, 1907) have been observed. They are transitory, disappearing during the fifth day of the chick's incubation period (*Remak*, 1855; *Platt*, 1889), if not before.

The segmentation of the rhombencephalon leads naturally to the question of metamerism of the nervous system. In the late nineteenth century, various investigators sought to homologize the development of the brain with the metameric development of the mesoderm, and several embryologists contended that the large primary brain vesicles were preceded by more primitive divisions of the neural tube. Since the spinal cord of the trunk region contains regular undulations opposite the somites, this suggestion gained considerable popularity. For a time it was widely accepted that the neural tube was "segmented" from end to end, and the concept of the descent of vertebrates from a segmented invertebrate ancestor was thought to have been strengthened.

It was claimed that the brain was derived from a number of segments (neuromeres) in the neural plate which could be seen anterior to the first somite before the formation of the neural folds. Various numbers of segments were counted, but eleven (*Locy*, 1895) was the number eventually most favored because of work done by *Hill* (1900). It was thought that fusion of these neuromeres formed the brain vesicles, although there was no unanimous agreement regarding the combinations of segments which produced the respective primary vesicles, as the accompanying table shows.

Investigator	Species	Forebrain	Midbrain	Hindbrain	Total
Béraneck (1887)	Chick ( <i>Gallus gallus</i> )	2	1	6	9
Platt (1889)	"	1	1	6	8
McClure (1890)	"	2	2	6	10
Zimmermann (1891)	"	2	3	8	13
Hill (1900)	"	3	2	6	11
Meek (1907)	Seagull ( <i>Larus</i> sp.)	3	2	13	18

encephali ventralis; 6, ganglion of the trigeminal (V cranial) nerve; 7, optic vesicle; 8, optic recess; 9, recessus neuroporicus; 10, otic vesicle; 11, sulcus telodiencephalicus; 12, sulcus mesodiencephalicus; 13, sulcus rhombomesencephalicus; 14, 15, acoustico-facialis ganglion complex; 16, interoptic furrow; 17, telodiencephalic eminence; 18, mesodiencephalic eminence; 19, rhombomesencephalic eminence; 20, parencephalon; 21, synencephalon; 22, optic stalk (or its stump); 23, metencephalon; 24, cranial flexure; 25, internal ridge between synencephalon and parencephalon; 26, infundibulum; 27, hypothalamus; 28, tuberculum posterius; 29, opening into optic stalk; 30, isthmus; 31, pars mammillaris hypothalami; 32, torus transversus; 33, ridge of optic chiasma; 34, optic cup and lens; 35, hemisphere; 36, epiphysis; 37, oculomotor nerve; 38, myelencephalon; 39, cervical flexure; 40, recessus mammillaris; 41, thalamus; 42, corpus striatum; 43, recessus infundibuli; 44, lamina terminalis; 45, ganglion of glossopharyngeal and vagus nerves; 46, roof plate of telencephalon medium; 47, mesencephalic tegmentum.



The concept of neural metamerism is no longer fully accepted. "Primitive" neuromeres anterior to the hindbrain usually are not revealed by improved techniques (Streeter, 1933; Kuhlenbeck, 1935), although O. Schumacher (1928) observed eleven very indistinct segments in the neural plate of the 1-somite lapwing (*Vanellus vanellus*) embryo. As Béraneck (1887) noted, the subdivisions in the rhombencephalon tend to gain in clarity for a time after their initial appearance, a fact that argues against their significance as primitive structures. Mihalkovics (1877, p. 49) suggested that the segments of the rhombencephalon were the mechanical result of the ventral curvature of the neural tube. It has also been said that they form in connection with the primordia of the cranial nerves (Remak, 1855; Béraneck, 1887).

**From the 15- to the 20-somite stage.** In addition to a general increase in diameter, the next most apparent changes in the brain are a longitudinal growth of the diencephalon and an upward expansion of its dorsal wall, as well as a continued forward and dorsal expansion of the telencephalon anterior to the optic vesicles. The external lateral view and the sagittal section of a 15-somite chick embryo presented in Fig. 93 reveal these changes. The telencephalon is divided from the diencephalon on either side of the sulcus telodiencephalicus, passing behind the optic vesicles (cf. Fig. 93-B<sub>1</sub>). The cavity of the telencephalon (soon to become plainly a bilobed structure) gives rise to the lateral ventricles.

The internal ridges which correspond to the external depressions, or sulci, between the brain vesicles are still visible. According to their respective locations, these ridges are known as the telodiencephalic, mesodiencephalic, and rhombomesencephalic eminences (cf. Fig. 93-B<sub>2</sub>).

The optic vesicles, until now communicating broadly with the forebrain cavity, begin to grow out on stalks which are directed slightly posteriorly (cf. Fig. 82-H). The openings into the optic vesicles from this time onward become progressively smaller. The lumina of the optic stalks open into a furrow, the sulcus interopticus, or interoptic groove. This groove crosses the inner surface of the anteroventral brain wall, passing through the optic recess, a depression in the midline (cf. Fig. 93-B<sub>2</sub>). A cross section through the forebrain region of a chick embryo of probably 16 or 17 somites (Fig. 94-C) shows the stalked structure of the optic vesicles, which now occupy a relatively more ventral position because of the dorsal growth of the forebrain. This illustration also shows that the forebrain appears relatively narrower for the same reason. A slight thickening of the ectoderm directly overlying the optic vesicles is the first indication of lens formation.

The ganglia of the trigeminal (V cranial) and acoustico-facial (VII-VIII cranial) nerves are conspicuous in the hindbrain (cf. Fig. 93-B<sub>1</sub>). The acoustico-facial ganglia are closely associated with the otic vesicles.



The cervical flexure is now evident, having started to appear at some time between the 13-somite stage (*O. Schumacher, 1928*) and the 15-somite stage (*Kamon, 1906*). The indentation produced by the incipient flexure on the ventral surface of the brain, between the diencephalon and the mesencephalon, is known as the *plica encephali ventralis*.

At the 20-somite stage, the relationships between the various parts of the brain have become considerably altered and several new structures have appeared. The cranial flexure is greatly accentuated (Fig. 93-C<sub>1</sub>), so that the axis of the anterior part of the brain now forms an acute angle with the axis of the hindbrain. Many investigators (*His, 1868, Kamon, 1906; O. Schumacher, 1928*) consider the cranial flexure to consist of an anterior and posterior flexure at the anterior and posterior boundaries, respectively, of the mesencephalon. The mesencephalon has been brought into such a position that it is now the most anterior portion of the brain, and the truly foremost parts are directed posteriorly. (In locating structures in the flexed brain tube, however, it is customary to orient them as if the brain were straightened out.)

All the brain vesicles have grown longitudinally as well as dorsad, their dorsal expansion being especially obvious in the sagittal section shown in Fig. 93-C<sub>2</sub>. The optic vesicles are now on distinct stalks directed dorso-posteriorly. The sulcus telodiencephalicus has become a more marked dorsolateral depression (cf. Fig. 93-C<sub>1</sub>). A cross section through the telencephalon (Fig. 94-D) shows the optic vesicles and reveals the fact that the cerebral hemispheres are bilateral almost from the beginning (*Baer, 1828b; Kamon, 1906; Kupffer, 1906*). The walls of the telencephalon are slightly thickened everywhere except dorsally, where the thin roof of the telencephalon medium is apparent; a slight furrow separates the roof plate from the lateral wall on either side. The early existence of the roof plate led *Henrich (1897)* to designate three divisions in the telencephalon.

The roof of the mesencephalon has elongated more than its floor. Between the midbrain and the diencephalon there is a seemingly constricted region (cf. Fig. 93-C<sub>1</sub>), the synencephalon (or *pars synencephalica diencephali*); this is considered another secondary brain vesicle. The portion of the brain heretofore known as the diencephalon is now usually renamed the parencephalon (or *pars parencephalica diencephali*). Although the synencephalon has been traditionally considered to be formed from the diencephalon, and therefore ultimately from the prosencephalon, it has also been classified as a derivative of the mesencephalon (*Kuhlenbeck, 1935*).

In the 20-somite chick, another slightly narrow portion of the brain tube is apparent just posterior to the midbrain. This region will later be more markedly constricted and will become the isthmus.

The roof of the rhombencephalon is perceptibly thinner than any other



portion of the brain wall. The most anterior part of the rhombencephalon, before tapering into the isthmus region, is very slightly expanded and less thinly roofed; it represents the earliest indication of the differentiation of the metencephalon. The remaining, posterior part of the rhombencephalon constitutes the early myelencephalon.

Further reference to Fig. 93-C<sub>2</sub> will reveal other structures not previously present on the internal surface of the brain wall. The ventrolateral wall of the parencephalon contains a longitudinal ridge corresponding to a groove on the external surface. The posterior end of this ridge is separated by a short groove from a small, rounder eminence. Together, these prominences constitute the primordial hypothalamus. A transverse ridge medial to the smaller prominence is the tuberculum posterius. Another transverse ridge on the dorsolateral wall of the brain tube marks the anterior limit of the synencephalon. Ventral to the early hypothalamus there is a slight evagination, the first outgrowth of the infundibulum, which is to enter into the formation of the hypophysis (sometimes considered part of the hypothalamus).

*From the 21- to the 50-somite stage.* As the number of somites increases from twenty to about twenty-seven, the development of the brain is chiefly characterized by the continued expansion of its larger chambers. The dorsal surface of the synencephalon bulges slightly. The internal openings of the optic stalks become continually smaller and, at about the 27-somite stage, communicate directly with the optic recess through the interoptic furrow. The hemispheres have grown laterally and dorsally, and the telodiencephalic eminence, or torus hemisphericus, which bounds each hemisphere posteriorly, has become more prominent internally.

The roof of the rhombencephalon, except in the metencephalic anlage and at the most posterior levels, is now very thin and appears epithelial in nature. When seen from either side, the transition from the thin roof to the thicker lateral wall appears as a sharp, concave line (*His, 1868, p. 130*).

At the 36-somite stage, the lateral bulging of the hemispheres is obvious, as may be seen in Fig. 94-E, which depicts a cross section taken through the telencephalon of a 36-somite chick in the region of the optic recess. The sulcus telodiencephalicus is much accentuated (Fig. 93-D<sub>1</sub>). According to O. Schumacher (1928), a temporary branch of this sulcus is noted in the 36-somite lapwing (*Vanellus vanellus*) embryo separating the dorsal portion of the parencephalon from the more ventral optic and infundibular regions. An increase in the height of the infundibular region is apparent at this time (cf. Fig. 93-D<sub>1</sub>). The oculomotor (III cranial) nerve has made its appearance; it can be seen issuing from the basal part of the mesencephalon (*Kamon, 1906; O. Schumacher, 1928*). The isthmus is starting to differentiate posterior to the mesencephalon, and, as a result, the posterior



cranial flexure is somewhat rounded off. The rhombencephalon has increased slightly in length. A sagittal section (Fig. 93-*D*<sub>1</sub>) clearly reveals the enlargement of the various brain vesicles, including the metencephalic region. In addition, a number of modifications are apparent on the internal surface of the brain. The ridges in the hypothalamic region have become two round prominences which will later form the pars mammillaris. Anterior to the enlarged infundibular evagination and immediately posterior to the optic recess is a transverse thickening, the early optic chiasma, transected by the sagittal plane of the section. Anterior to the optic recess is a similar transverse thickening, the torus transversus, continuous with the torus hemisphericus. The torus transversus marks the location of the future anterior commissure.<sup>1</sup> In the lateral wall of the rhombencephalon, six neuromeres can easily be seen.

By the 36-somite stage, the cervical flexure is plainly visible (*His*, 1868, *p.* 131; *Kamon*, 1906; *O. Schumacher*, 1928). This is a curvature, with its convexity directed dorsally, at the juncture of the rhombencephalon and the spinal cord.

At the 40-somite stage, the increase in the size of the mesencephalon is the most striking change. The next most obvious development is the increasing prominence of the metencephalic region posterior to the isthmus (Fig. 93-*E*<sub>1</sub>). The isthmus is now a relatively narrow constriction between the neighboring parts of the brain. The hemispheres have grown considerably, chiefly in the dorsal direction, and appear as rounded structures. The sulcus telodiencephalicus crosses the dorsal surface of the brain and proceeds down the lateral surfaces, passing anterior to the optic stalks rather than posterior to them, as formerly. A small process, the epiphysis, projects from the roof of the parencephalon. The oculomotor nerve can be seen leaving the ventrolateral wall of the mesencephalon. An internal view (Fig. 93-*E*<sub>2</sub>) of the brain at this period shows the increased thickness of the optic chiasma and the ridge which runs dorsally from it. The torus transversus is also thicker. The primordium of the corpus striatum is apparent as a swelling of the lateral brain wall dorsal to the torus transversus. The torus hemisphericus is thin in the dorsal midline and is here termed the velum transversum. The more anterior prominence of the pars mammillaris hypothalami has increased in size considerably. Dorsal and anterior to the pars mammillaris can be seen two eminences, the first indications of the thickening of the diencephalic wall which will later produce the thalamus. The infundibulum has evaginated farther in the anterior direction to form the postoptic recess, or recessus infundibuli, and in the posterior direction to form the recessus mammillaris. The ridge anterior to the synencephalon is becoming limited to the dorsal portion of this part of the brain. According to *Cohen and Davies* (1937), a small median area of flat-

<sup>1</sup> Commissure: a band of nerve tissue connecting the two sides of the brain.



tenced cells in the roof of the myelencephalon has become sharply demarcated from the surrounding epithelium.

The brain of the canary (*Serinus canaria*) embryo at approximately this stage of development may be seen in Fig. 95-A<sub>1</sub> and A<sub>2</sub>. A diagrammatic sagittal section (Fig. 96-A) shows the brain in relation to the other tissues of the cranial region.

A dorsal view of the 40-somite chick embryo's brain appears in Fig. 100-A, which clearly indicates that the round hemispheres are bilateral

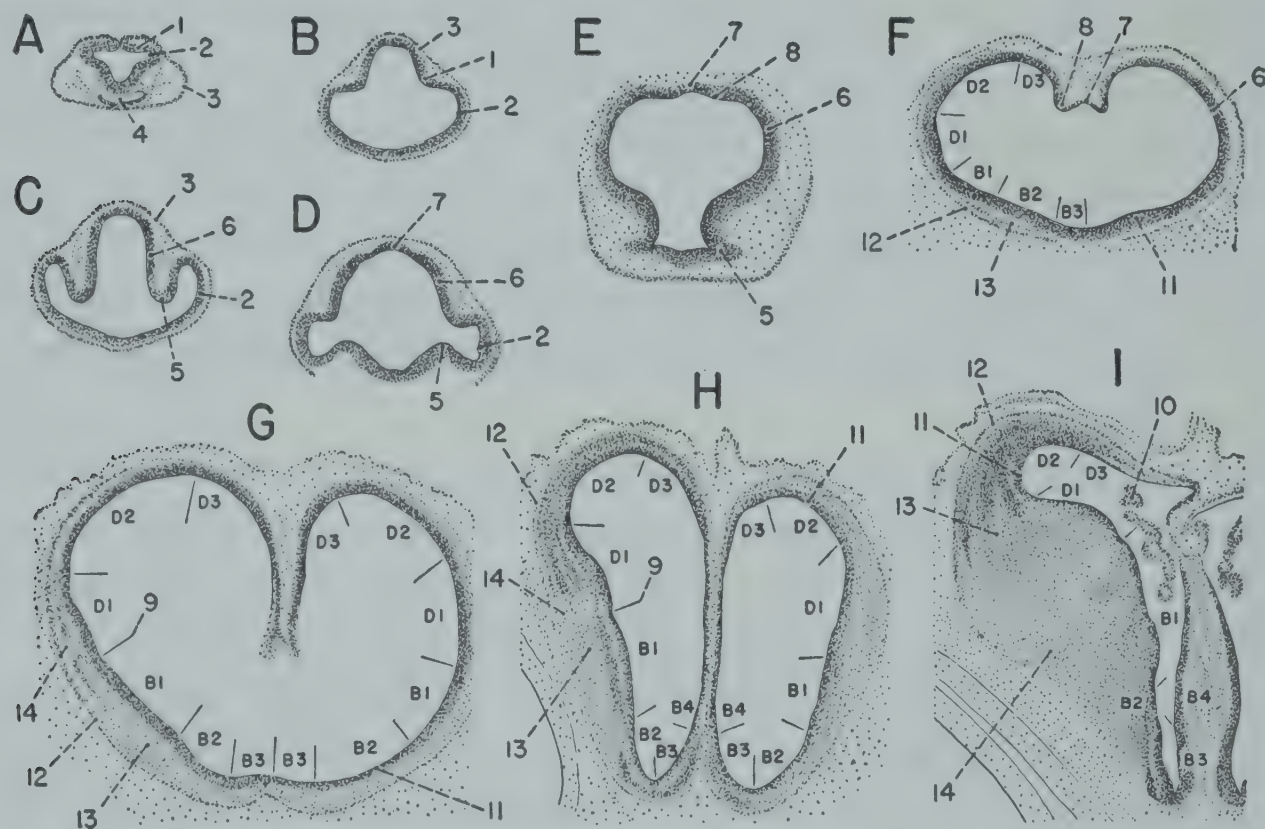


Fig. 94. The development of the telencephalic region of the chick embryo's brain, as shown by cross sections taken at various times from the first to the seventh day of incubation. All  $\times 310$ . (Redrawn with modifications A, after His, 1868; B and C, after Kessler, 1877; D, after Kamon, 1906; E, after Kupffer, 1906; F, G, H, and I, after Kuhlenbeck, 1938.)

1, neural tube; 2, optic evagination; 3, surface ectoderm; 4, fore-gut; 5, optic stalk; 6, forebrain or hemisphere; 7, telencephalon medium and roof plate; 8, furrow; 9, ventricular groove; 10, chorioid plexus; 11, germinal or ependymal layer; 12, marginal layer; 13, mantle layer; 14, zona limitans.

The basal and dorsal zones are shown by B1-B4 and D1-D3, respectively.

structures rising high on either side of the median portion of the telencephalon (telencephalon medium). A cross section through the telencephalon, represented in Fig. 94-F reveals that the roof of the telencephalon medium is much thinner than the walls of the hemispheres, and that it is somewhat elevated in the midline. This section also shows that the vertebrate pattern of longitudinal zones, separated by limiting sulci, is beginning to become apparent. Six zones, three basal and three dorsal, are present and are respectively designated in the figure as B1, B2, B3, and D1, D2, and D3.



It is generally accepted that the depression of the telencephalon medium between the hemispheres is merely the result of the enlargement of the latter structures, whose bilaterality is the expression of an inherent growth process (*Baer, 1828b; Kamon, 1906; Kupffer, 1906*). However, other explanations have been offered. *His (1868, p. 131)* suggested that the axial connection of the infundibulum with the anterior end of the fore-gut created a tension which prevented the growth of the telencephalon medium, although *Kupffer (1906)* indicated that this connection is lost in birds before the development of the hemispheres begins. *Mihalkovics (1877, p. 105)* attributed the bilaterality of the hemispheres to the active ingrowth of the connective tissue of the falx cerebri in the midline.

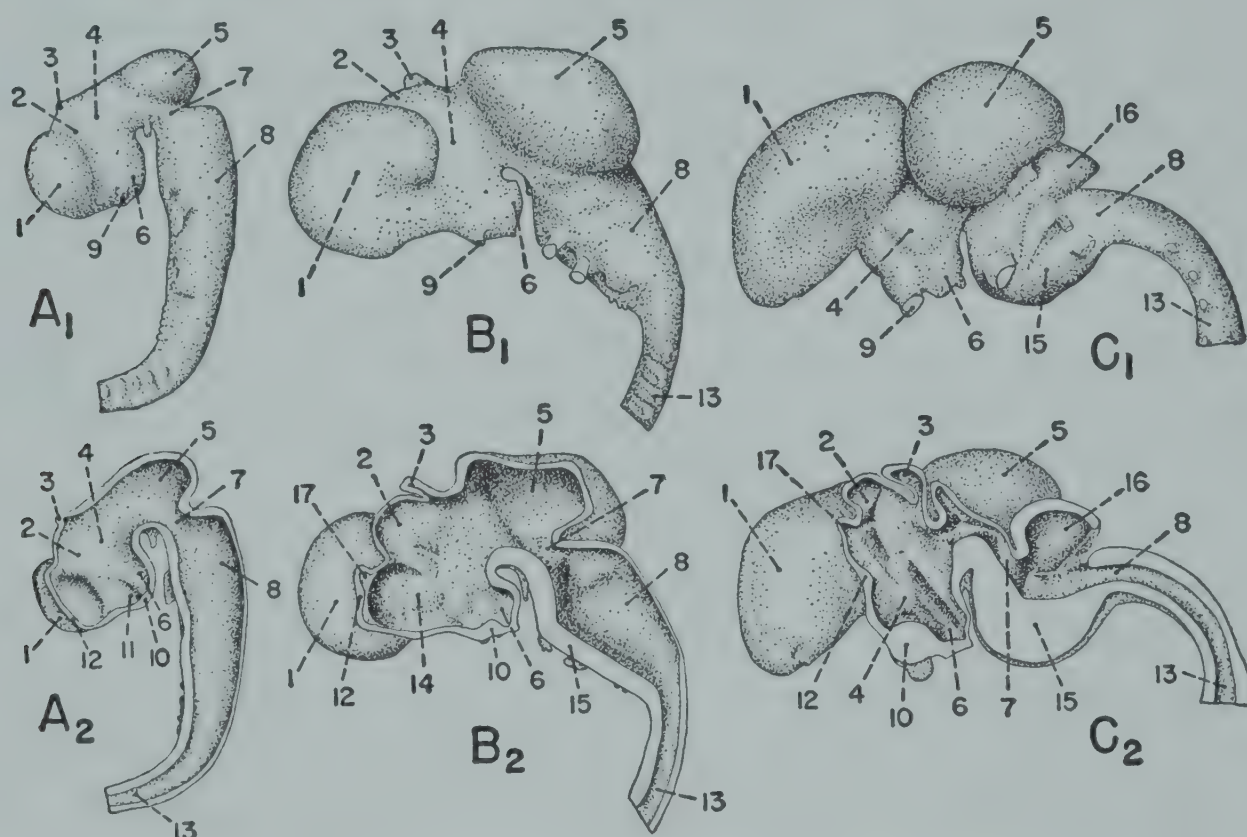
The cranial and cervical flexures are at their maximum in avian embryos of about this stage of development (*Kamon, 1906; O. Schumacher, 1928*).

At the 50-somite stage, found in the chick of 4 to 4.5 days' incubation, it is evident that the growth of the hemispheres is now in a caudal direction and that their posterior edges overlap the wall of the parencephalon (Fig. 93-*F*<sub>1</sub>). When seen from above, the hemispheres are no longer circular in outline but oval, and they diverge from each other posteriorly (cf. Fig. 100-*B*). The telencephalon medium is barely visible between them. The epiphysis is a hollow, stalked structure directed cranially and, due to the growth of the parencephalon anterior to its point of attachment, appears to have shifted caudally (cf. Fig. 93-*F*<sub>1</sub>). The synencephalon is of noticeably greater length than previously. The mesencephalon has also undergone a tremendous enlargement in all directions, especially laterally and posteriorly, and projects over the metencephalon in a caudal direction. It resembles a large bubble. The isthmus appears very narrow. The metencephalon and myelencephalon are enlarged, but no external division is yet apparent between them. The cranial nerve ganglia present in the hindbrain are much more prominent. The otic vesicle is well defined. At this stage, the angle between the longitudinal axes of the anterior brain and the hindbrain is starting to become less acute.

A sagittal section through the brain (Fig. 93-*F*<sub>2</sub>) shows that the optic (preoptic) recess is sunk so deeply in a depression between the considerably thickened optic chiasma and the primordial corpus striatum that it projects as an obvious ridge on the external surface. The anterior sulcus intraencephalicus can be seen proceeding dorsally out of the optic recess. The corpus striatum forms a prominence that outlines the ventral side of the opening into each hemisphere (the foramen of Monro) and merges dorsally with the telodiencephalic eminence (torus hemisphericus). A very slight dorsal evagination, forerunner of the paraphysis, is beginning to form in the telencephalon medium, which has grown very thin, especially anterior to the evagination. The two structures forming the pars mammil-



laris hypothalami have fused together, and the recessus infundibuli and the recessus mammillaris are more pronounced. A prominence in the floor of the midbrain is the developing mesencephalic tegmentum, delimited dorsolaterally by a longitudinal anterior continuation of the sulcus lateralis internus on the ventrolateral wall of the metencephalon. The neuromeres in the myelencephalon are barely perceptible. The sharply demarcated membranous portion of its roof is larger than before (*Cohen and Davies, 1937*).



**Fig. 95.** The brain of the canary (*Serinus canaria*) embryo. External (left side) and internal (right side) views at three stages. (Redrawn with modifications after Boss, 1913.)

A, B, and C, correspond to the stages found in the chick embryo at 3.5 days, 5 days, and 8 to 9 days of incubation, respectively. All  $\times 6$ .

1, hemisphere; 2, parencephalon; 3, epiphysis; 4, diencephalon; 5, mesencephalon or optic lobe; 6, infundibulum; 7, isthmus; 8, rhombencephalon; 9, optic stalk; 10, optic chiasma; 11, optic recess; 12, lamina terminalis; 13, spinal cord; 14, corpus striatum; 15, pontine flexure; 16, cerebellum; 17, paraphysis.

A very slight outward bulging of the ventral wall of the myelencephalon represents the first faint indication of the onset of the pontine flexure. The cervical flexure is starting to become slightly less pronounced. Figure 96-B is a diagrammatic sagittal section through the head of the canary (*Serinus canaria*) embryo at this stage of development and shows the relationship of the brain to other structures.

A cross section through the telencephalon of a slightly older (5-day) chick embryo appears in Fig. 94-G. The thickening of the wall on the left side (the section is slightly oblique) represents the early corpus striatum.

**The final stage of the early phase.** The stage of development corresponding to that found in the chick's brain at about 5 to 5.5 days' incuba-



tion is shown in Fig. 95-B<sub>1</sub> and B<sub>2</sub>, which depict the brain of the canary (*Serinus canaria*) embryo. The external view, Fig. 95-B<sub>1</sub>, reveals several noteworthy changes. There is a considerable increase in the mass of all portions of the brain, but particularly of the myelencephalon. The angle formed between the forebrain and hindbrain axes is now obtuse. The infundibular region has been brought almost into contact with the ventral wall of the myelencephalon, and the optic region, together with the optic nerve, has grown posteriorly. The pontine flexure is beginning to cause a ventrally directed convexity in the floor of the hindbrain; and the sagittal section, Fig. 95-B<sub>2</sub>, shows that this flexure is the result, to a large extent,

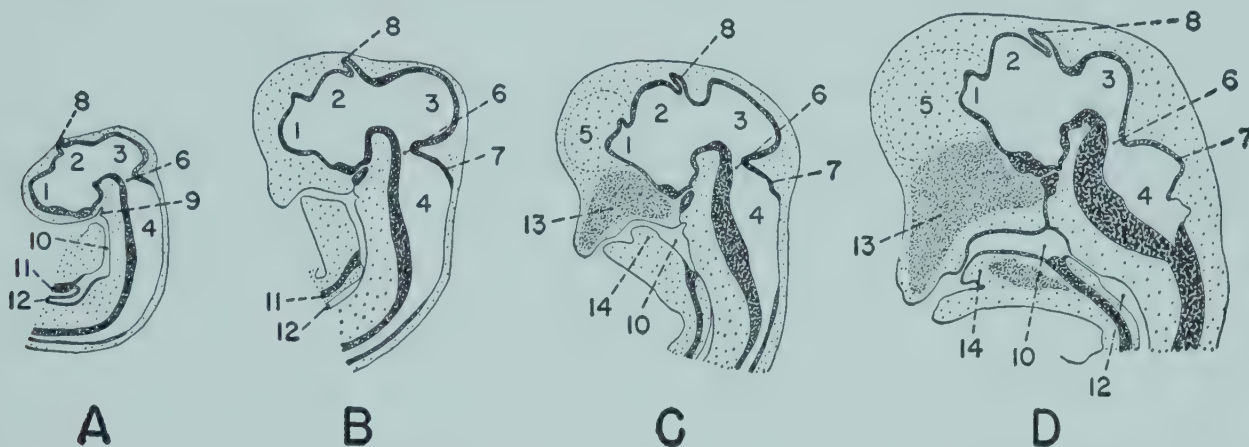


Fig. 96. Changes in the relationship of the brain to the remaining structures of the head during embryonic development, shown by diagrammatic sagittal sections through the head of the canary (*Serinus canaria*) embryo. (Redrawn with modifications after Boss, 1913.)

A, stage approximating that found in the chick (*Gallus gallus*) embryo at 3 to 4 days; B, the same, at 4 to 5 days; C, the same, at 5 to 6 days; D, the same, at 8 days. All  $\times 4.5$ .

1, telencephalon; 2, diencephalon; 3, mesencephalon; 4, rhombencephalon; 5, hemisphere; 6, isthmus; 7, metencephalon; 8, epiphysis; 9, hypophysis; 10, pharynx; 11, trachea; 12, esophagus; 13, interorbital septum; 14, tongue.

of the greatly increased thickness of the floor of the myelencephalon in the region of the future pons Varolii. This thickening of the basal region extends anteriorly through the midbrain and the synencephalon and finally is lost in the infundibular region. There is a slight indication of a transverse fold in the roof of the myelencephalon just posterior to the median membranous area, which has started to decrease in size (Cohen and Davies, 1937). A thickening of the lateral walls of the metencephalon can be seen at this age (Mesdag, 1909). The epiphysis is longer, and the paraphysis is plainly apparent at the summit of an elevation in the roof of the telencephalon medium. The optic chiasma is greatly accentuated, and the preoptic and postoptic recesses thereby more apparent. The corpus striatum can be seen as a prominence partially occluding the foramen of Monro. The posterior, bulging portion of the mesencephalon now possesses a median wall, and it is evident that the bilateral optic lobes (corpora bigemina) are coming into existence. According to Tello (1923),



the mesencephalon begins to divide on the fifth day, division being accomplished from posterior to anterior by the formation of a furrow in which a septum develops. The bilobate character of the mesencephalon at this stage is better seen in the dorsal view presented in Fig. 100-C. This illustration also shows that the hemispheres have grown posteriorly as well as anteriorly.

The position which the brain occupies within the head of the canary (*Serinus canaria*) embryo at approximately this stage of development is shown in Fig. 96-C. It may be observed that a small stomodaeal diverticulum (Rathke's pouch) is applied against the infundibular region. This diverticulum will later form the pars buccalis of the hypophysis cerebri.

### *The Second Phase of Development*

During the second phase of development which the chick embryo's brain enters at 5 to 6 days' incubation, the most pronounced changes in morphology are apparent on the ventricular surfaces and in cross sections of the brain, rather than externally. These changes are due to the increasing evolution of definitive cytoarchitecture.

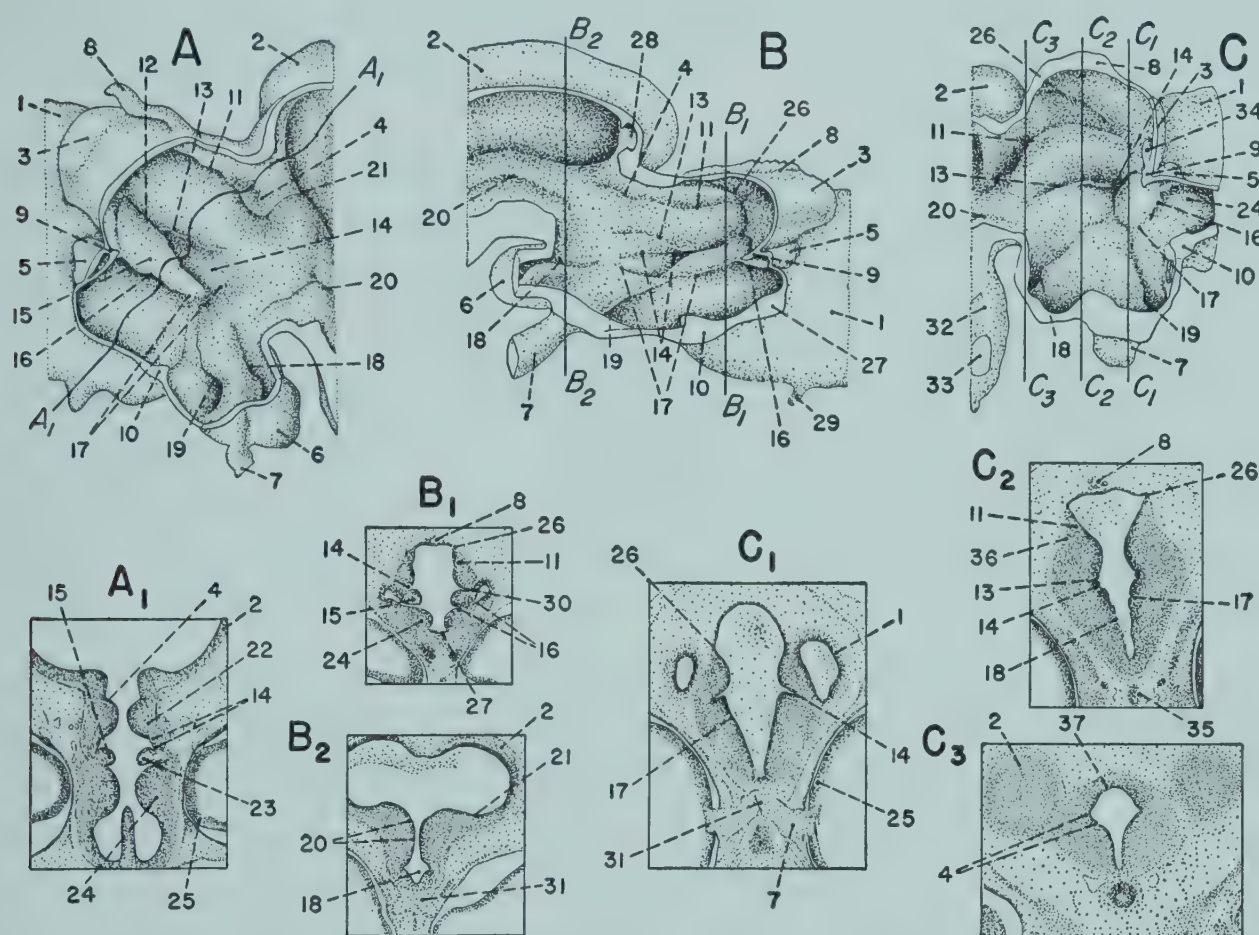
The advance in the organization of the avian diencephalon into longitudinal zones is shown in Fig. 97-A, which is a large-scale internal view of this region in the chick embryo of 5 to 6 days' incubation. The left side of the brain has been removed posterior to the root of the epiphysis. Conspicuous in the posterior parencephalic region are two obliquely longitudinal eminences that taper ventroposteriorly. The more dorsal is the pars dorsalis thalami. The more ventral is the eminentis thalami ventralis, occupying the place of the torus hemisphericus. Between these two structures of the thalamus lies the longitudinally coursing sulcus parencephalicus, which has the form of a fan-shaped groove, or fossa, with apex directed anteriorly. From this apex a deep, narrow groove, the anterior sulcus diencephalicus medius, continues in an anterior direction between the dorsal and ventral thalamic prominences. At the dorsal boundary of the sulcus parencephalicus, another narrow groove, the posterior sulcus diencephalicus medius, outlines the ventral border of the dorsal thalamus. These two branches of the sulcus diencephalicus medius will gradually replace the sulcus parencephalicus. The cell masses in the ventral thalamic eminence and in the depth of the sulcus parencephalicus, as far dorsal as the posterior sulcus diencephalicus medius, constitute the pars ventralis thalami.

Other sulci are apparent in this figure. These are the sulcus diencephalicus dorsalis, between the dorsal thalamus and the epithalamus; the sulcus terminalis, between the ventral thalamic eminence and the corpus striatum; the sulcus diencephalicus ventralis, continuous with the sulcus terminalis and curving upward into the sulcus parencephalicus; the anterior sulcus



intraencephalicus and the internal sulcus lateralis infundibuli, forming, respectively, the anterior and posterior limits of the ridge of the optic chiasma; the sulcus limitans, on the lateral wall of the basal mesencephalic and synencephalic regions; and the sulcus lateralis mesencephali, curving dorsoposteriorly into the optic lobe from the small depression which is all that remains of the synencephalic vesicle.

In Fig. 97-A, a protuberance seen on the basal surface of the right hemisphere is the early olfactory bulb. The paraphysis appears as a pocket in the midline directly anterior to the velum transversum.



**Fig. 97.** The diencephalic region of the brain of the chick embryo of various ages, as seen in views of the ventricular surface and in cross sections. (Redrawn with modifications after Kuhlenbeck, 1936.)

A, interior of the diencephalon at 5 days 20 hours of incubation; B, the same, 6 days; C, the same, 7 days. In A, most of the left side of the brain has been removed, and in B and C, the right side has been excised. A<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, cross sections taken at the levels indicated by lines in A, B, and C. All  $\times 5.5$ .

1, hemisphere; 2, optic lobe; 3, parencephalon; 4, synencephalon; 5, paraphysis; 6, infundibulum; 7, optic nerve; 8, epiphysis; 9, velum transversum; 10, torus transversus; 11, sulcus diencephalicus dorsalis; 12, sulcus diencephalicus medius; 13, posterior part of sulcus diencephalicus medius; 14, sulcus parencephalicus; 15, ventral thalamic eminence; 16, sulcus terminalis; 17, sulcus diencephalicus ventralis; 18, sulcus lateralis infundibuli; 19, sulcus intraencephalicus anterior; 20, sulcus limitans; 21, sulcus lateralis mesencephali (internus); 22, dorsal thalamus; 23, torus hemisphericus; 24, corpus striatum; 25, eye; 26, sulcus taeniae thalami (internus); 27, lamina terminalis; 28, postcommissural recess; 29, olfactory nerve; 30, sulcus telodiencephalicus; 31, optic chiasma; 32, medulla; 33, trigeminal nerve; 34, chorioid plexus; 35, hypophysis; 36, sulcus diencephalicus dorsalis externus; 37, posterior commissure.



An oblique cross section taken at this stage through the mesencephalon, the thalamic region, the corpora striata, and the foramina of Monro is pictured in Fig. 97-A<sub>1</sub>. This section reveals the increased thickness of the brain wall in well-defined regions, and shows the varying depth of the sulci delimiting these regions. (The right side of the section is taken slightly more posteriorly than the left.)

The thickening of the brain wall and the accentuation of the longitudinal zones can be followed in the hemispheres also. In Fig. 94-H, which is a section through the rostral portion of the telencephalon of the 5- to 6-day chick, the lateral wall in the zones B1 and D1 is greatly thickened. (The plane of section is not perpendicular to the long axis.) The limiting sulci are still evident, and a new zone, B4, has differentiated on the medial wall of each hemisphere.

The thickened lateral walls of the metencephalon are now recognizable as the paired anlagen of the cerebellum. A slight depression on the lateral surface of each swelling separates the primordial auricles from the region that represents the future corpus cerebelli (*Larsell, 1948*).

After 6 days of incubation, the diencephalic region has undergone considerable longitudinal growth and its internal aspect has changed, as Fig. 97-B shows. The sulcus taeniae thalamis internus can be seen in the roof of the parencephalon, delimiting the epithalamus dorsally. The sulcus diencephalicus dorsalis, forming the ventral boundary of the epithalamus, is now continuous with the small synencephalic recess. The posterior sulcus diencephalicus medius has extended farther posteriorly, and the sulcus parencephalicus lies removed from it in a ventral direction and is disappearing. At the junction of the roof of the mesencephalon with that of the synencephalon, a thickening of the wall represents the posterior commissure.<sup>1</sup> Above this lies the diverticulum-like recessus postcommissuralis (or supracommissuralis). The tectal commissure<sup>2</sup> projects slightly, dorsal to the recessus postcommissuralis. Another newly thickened portion of the brain wall lies in the lamina terminalis anterior to the torus transversus and indicates the location of the future pallial commissure. The portion of the torus hemisphericus lying between the torus transversus and the ventral thalamic eminence has completely disappeared, due to the continued growth of the corpus striatum. The sulcus terminalis, dorsal to the corpus striatum, leads through the foramen of Monro into the lateral ventricle.

Figure 97-B<sub>1</sub> is a cross section through the parencephalic region at a point more or less corresponding to that at which Fig. 97-A<sub>1</sub> was taken. (The optic lobes are not transected because of the longitudinal growth of the diencephalic region in the intervening period.) The left side of the

<sup>1</sup> The posterior commissure connects the optic lobes.

<sup>2</sup> The fibers of this commissure pass across the roof of the aqueduct of Sylvius.



section passes through the foramen of Monro and the right side passes slightly more posteriorly. The great increase in the size of the corpora striata is apparent. A cross section taken much farther posteriorly is shown in Fig. 97-B<sub>2</sub>. This section cuts through the optic chiasma and the optic lobes, the optic nerves leading into the optic chiasma from either side.

After about 6 to 6.5 days' incubation, the brain of the chick has reached a stage of development approximately corresponding to that shown in Fig. 99-A which represents the brain of the embryonic lapwing (*Vanellus vanellus*). The hemispheres have increased considerably in length and extend sufficiently far posteriorly to hide a large portion of the parencephalon. On the lateral surface of each hemisphere there is a depression

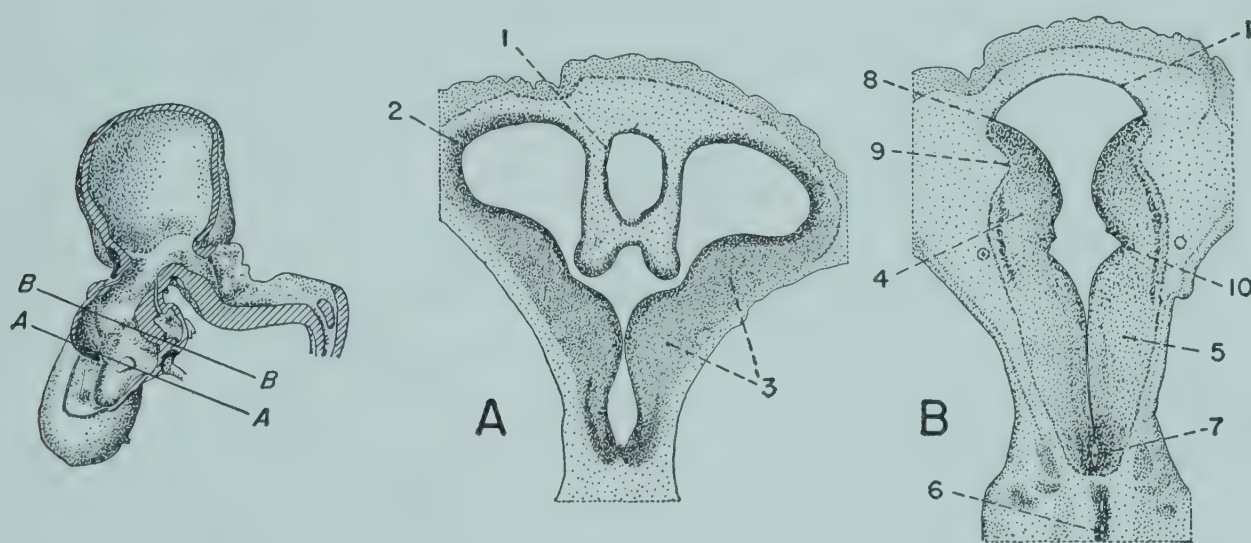


Fig. 98. Cross sections through the brain of the lapwing (*Vanellus vanellus*) embryo at a stage approximately equivalent to the 6.5-day stage in the chick embryo's brain. (Redrawn with modifications after O. Schumacher, 1928.)

A, a cross section through the hemispheres and the parencephalic region of the brain; B, a cross section through the thalamic and infundibular regions. Both  $\times 12$ .

At left: a left lateral view of the right half of the brain after excision of the left half, with the levels of sections A and B indicated by lines ( $\times 4$ ).

1, parencephalon; 2, telencephalon; 3, corpus striatum; 4, thalamus; 5, hypothalamus; 6, hypophysis; 7, lumen of the infundibulum; 8, sulcus parencephalicus internus; 9, sulcus parencephalicus externus; 10, sulcus parencephalicus transversus.

into which fits the now greatly enlarged eye. Near the anterior pole of the hemisphere the olfactory nerve is given off basally. On the outer posterior wall of the infundibular region can be seen a small prominence, the tuberculum mammillare. The optic lobes have grown so that they overlap the median portion of the metencephalon. The lateral walls of the metencephalon are thickening noticeably. The pontine flexure has become more pronounced.

An internal view of the lapwing's (*Vanellus vanellus*) brain at this stage (cf. Fig. 99-A) shows that, in this species, the right and left halves of the brain have made contact with each other in several places. A small area of contact is found between the corpora striata, which have continued to



increase in thickness. These structures practically occlude the foramina of Monro. Each corpus striatum consists of a dorsal and a basal portion divided by a longitudinal furrow, which is a continuation of the sulcus terminalis (Kuhlenbeck, 1936). A cross section through the hemispheres and the anterior part of the parencephalon is represented in Fig. 98-A, and shows these divisions of the corpora striata. Figure 98-A also shows that the roof of the telencephalon medium is elevated in the midline and that the medial walls of the hemispheres are very thin.

Another large area of contact between the two sides of the lapwing's (*Vanellus vanellus*) brain is found in the region of the hypothalamus (cf. Fig. 99—Insert *a*), posterior to which there remains only a very narrow communication between the lumen of the infundibular region and that of the mesencephalic region. The cross section in Fig. 98-B is taken through the dorsal thalamus and the hypothalamus at a level where the right and left halves of the latter structure are in contact with each other.

Certain other changes are apparent in Fig. 99-A. Between the hemispheres can be seen a short fold, the primordium of the chorioid plexus of the (left) lateral ventricle. The cerebellar commissure and the decussation of the trochlear (IV cranial) nerve have developed at the dorsal junction of the mesencephalon and the metencephalon, and the inferior commissure (postoptic commissure) can be seen immediately posterior to the optic chiasma. The sulcus lateralis isthmi continues into the hindbrain as the sulcus lateralis rhombencephali, dividing the floor from the cerebellar anlage. Lateral to the cerebellar primordium the transverse sulcus has extended to the lateral wall, further delimiting the cerebellum. In the chick of equivalent developmental age, the membranous area anterior to the transverse sulcus is very small and a similar membranous area has begun to differentiate posterior to the sulcus (Cohen and Davies, 1937).

In the chick of 6.5 to 7 days' incubation, a shift in the relative positions of the various parts of the brain is increasingly evident. The hemispheres and the optic lobes, growing steadily, have been brought closer together dorsally. The cranial flexure has become less pronounced and the pontine flexure accentuated, and thus the longitudinal axis of the brain has been straightened somewhat. As a consequence of these changes, the entire diencephalic region, including the infundibulum, has suffered a decrease in anteroposterior length (Huber, 1949*b*), and its structures have undergone a directional alteration.

A sagittal section of the brain of the lapwing (*Vanellus vanellus*) embryo at a similar stage of development is depicted in Fig. 99-B. Although the dorsal portions of the hemispheres have grown considerably, mainly in the posterior direction, the most conspicuous increase in size is along the basal surface. In consequence, the olfactory bulb and nerve are no longer ventral in position but occupy the forward apex of each hemisphere, and



the hemispheres themselves appear to taper to a point anteriorly. Their medial surfaces are flat. Their lateral walls have continued to thicken, as Fig. 94-I shows. Anterior to the region shown in Fig. 94-I, zones B1 and B2 become much smaller and zone D1 extends much farther basally (Kuhlenbeck, 1938).

As the diencephalic region is gradually overgrown anteriorly and posteriorly by the hemispheres and optic lobes, respectively, it further de-

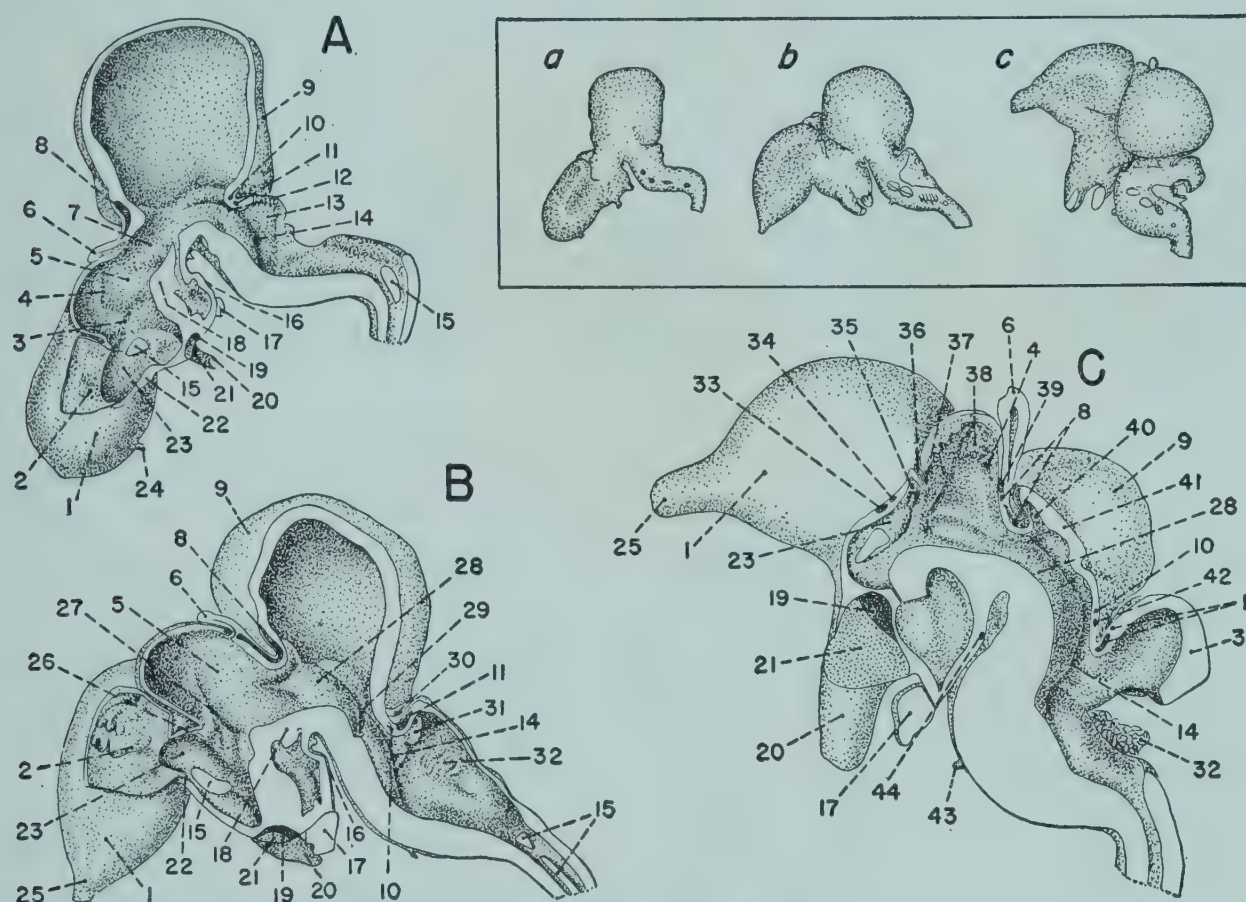


Fig. 99. The brain of the European lapwing (*Vanellus vanellus*) embryo. (Redrawn with modifications after O. Schumacher, 1928.)

A, stage approximating that found in the chick (*Gallus gallus*) embryo at 6 days' incubation; B, the same, at 7.5 days; C, the same, at 10 days. The left side of the brain has been removed. All  $\times 5$ .

Insert: external lateral views of the left side of the brain at stages *a*, *b*, and *c*, corresponding to those shown in A, B, and C. All  $\times 2$ .

1, hemisphere; 2, chorioid plexus of lateral ventricle; 3, telodiencephalic eminence; 4, sulcus parencephalicus longitudinalis; 5, thalamus; 6, epiphysis; 7, synencephalon; 8, posterior commissure; 9, optic lobe; 10, decussation of trochlear (cranial IV) nerve; 11, cerebellar commissure; 12, sulcus lateralis isthmi; 13, lamina cerebellaris; 14, sulcus lateralis rhombencephali; 15, area of contact with other side of brain; 16, tuberculum mamillare; 17, hypophysis; 18, hypothalamus; 19, Gudden's commissure; 20, optic nerve; 21, optic chiasma; 22, torus transversus; 23, corpus striatum; 24, olfactory nerve; 25, olfactory bulb; 26, roof of foramen of Monro; 27, sulcus parencephalicus transversus; 28, mesencephalic tegmentum; 29, sulcus intraencephalicus posterior; 30, trochlear (cranial IV) nerve; 31, cerebellum; 32, chorioid plexus of fourth ventricle; 33, anterior commissure; 34, commissura pallii anterior; 35, foramen of Monro; 36, paraphysis; 37, velum transversum; 38, chorioid plexus of third ventricle; 39, habenular commissure; 40, recessus postcommissuralis; 41, lamina commissuralis; 42, commissura veli; 43, abducent (cranial VI) nerve; 44, commissura infundibuli.



creases in length and gains in height and breadth. The longest diameter of the infundibular region then parallels the long axis of the hindbrain.

Figure 97-C gives an internal view of the left side of the diencephalon of the chick embryo of 7 days' incubation. The lamina terminalis, from the preoptic recess to the torus transversus, rises almost perpendicularly. Many of the sulci that formerly ran longitudinally now run transversely, especially the sulcus lateralis infundibuli, the sulcus intraencephalicus anterior, the sulcus terminalis, and the sulcus parencephalicus. The sulcus parencephalicus is greatly shortened and much deeper, except for a shallow longitudinal branch running posteriorly and almost coinciding with the posterior sulcus diencephalicus medius. The sulcus diencephalicus dorsalis is no longer straight, but curves downward posteriorly. The synencephalic recess cannot be distinguished. A fold of the chorioid plexus of the left lateral ventricle appears in section in Fig. 97-C, and the paraphysis, not sectioned, can be seen in the midline anterior to the parencephalon, whose anterior wall is shown intact.

Several cross sections are shown to clarify the relationships between the various structures and sulci of the diencephalon. Figure 97-C<sub>1</sub> represents a section taken anterior to the epiphysis and transecting the optic chiasma and the posterior part of the hemispheres. The optic nerves can be seen leading to the eyes. The thin, bulging roof of the parencephalon and the depth of the sulcus parencephalicus are to be noted. A section taken farther posteriorly, approximately through the middle of the parencephalon, appears in Fig. 97-C<sub>2</sub>. Here both epiphysis and hypophysis are transected. The roof of the parencephalon is almost flat, the sulcus parencephalicus is small and shallow, and the lumen of the brain is narrow basally. (In the chick embryo of this stage, contact between the walls of the diencephalon has not been made as it has in the lapwing (*Vanellus vanellus*)). Figure 97-C<sub>3</sub> shows a still more posterior section taken just anterior to the isthmus. The anterior walls of the optic lobes appear in this section, which passes through the tuberculum mammillare of the infundibulum and the beginning of the posterior commissure. The very much reduced synencephalic recess is shown.

The optic lobes and the hemispheres have continued to enlarge. The separation of the optic lobes is complete by the seventh day, and their anterior poles then start to diverge from each other (*Tello, 1923*) as the posterior poles of the hemispheres draw nearer (cf. Fig. 100-D).

The anterior wall of the metencephalon, the lamina cerebellaris, rises almost perpendicularly behind the isthmus (cf. Fig. 99-B). The cerebellar swellings protrude into the fourth ventricle but have not yet met in the midline. The increased curvature of the pontine flexure has brought about a shortening of the thin roof of the hindbrain, the lamina epithelialis, which, in the dorsal aspect, is diamond-shaped. The early chorioid plexus



of the fourth ventricle is starting to form on the internal surface of the lamina epithelialis (O. Schumacher, 1928).

At 8 days of incubation, the brain of the chick appears much like that of the canary (*Serinus canaria*) embryo's brain shown in Fig. 95-C<sub>1</sub> and C<sub>2</sub>. The paired cerebellar swellings are separated by a narrow median groove. The depression setting off the extreme lateral tip of each cerebellar swelling is starting to extend medially as a slight furrow. This stage of cerebellar development is found in the 10-day embryo of the duck, *Anas platyrhynchos* (Larsell, 1948). In the 8-day chick embryo, the membranous area

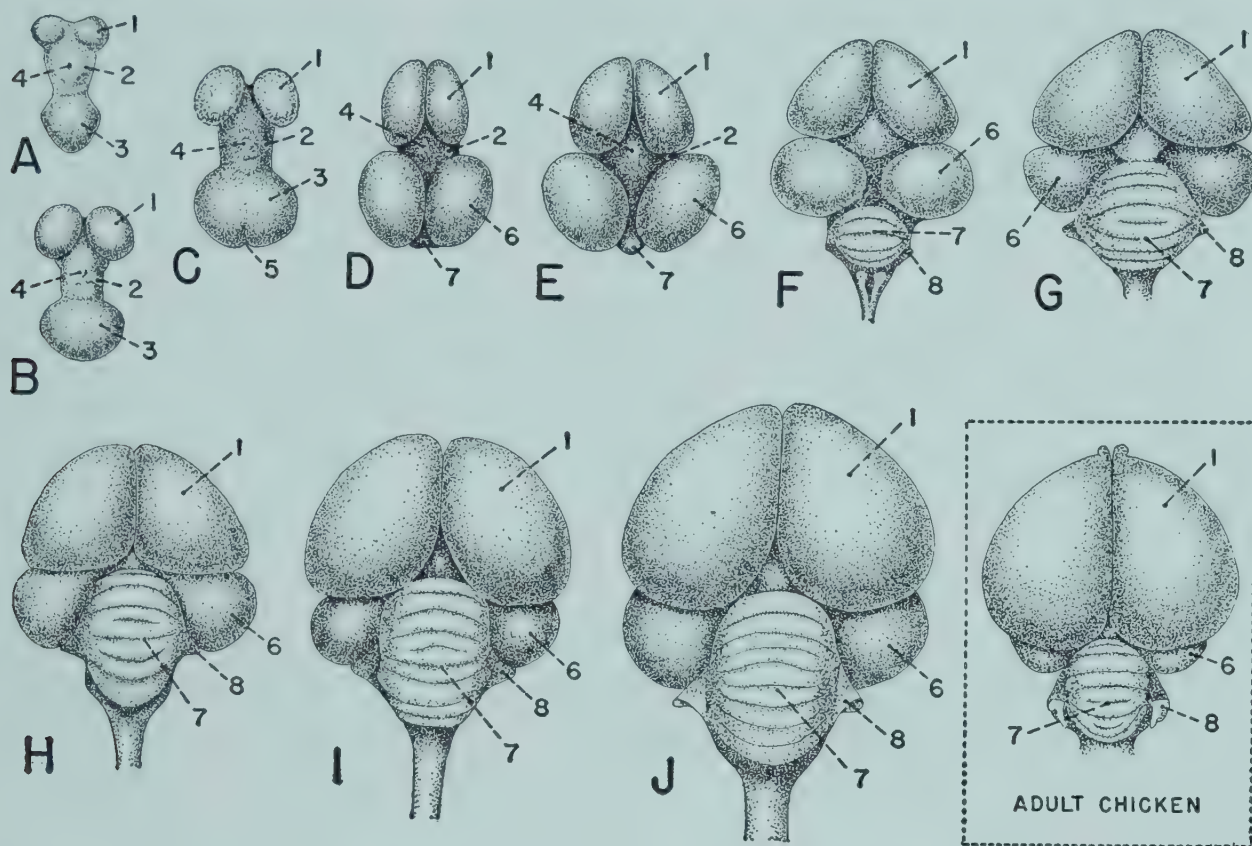


Fig. 100. The progressive development of the chick embryo's brain, as seen in dorsal view. (Redrawn with modifications after Kamon, 1906; Oliver, 1912; Huber, 1949a, 1949b.)

A, the brain shown at 3 to 4 days' incubation; B, 4 to 5 days; C, 5 days; D, 7 to 8 days; E, 9 to 10 days; F, 12 days; G, 14 days; H, 16 days; I, 18 days; J, 20 days. All  $\times 2$ .

Insert: the brain of the adult chicken (natural size).

1, hemisphere; 2, diencephalon; 3, mesencephalon; 4, epiphysis; 5, early median sulcus in mesencephalon; 6, optic lobe; 7, cerebellum; 8, flocculus.

posterior to the transverse sulcus of the hindbrain has enlarged to such an extent that it occupies the entire roof of the fourth ventricle. At the 8.5-day stage, the very small anterior membranous area is incorporated into the anterior wall of the transverse sulcus (Cohen and Davies, 1937).

After 8 days of incubation, the thickening of the walls of the chick's hemispheres extends to the lateral part of the dorsal zone D2 and to the basal zones B3 and B4 on the medial walls, the early stages of which are shown in Fig. 94. The cross section of the telencephalon has assumed an unmistakably avian appearance (Kuhlenbeck, 1938).



On or before the chick's ninth day of incubation, the optic lobes and the hemispheres make contact with each other in the diencephalic region, the greatest portion of which is then invisible when the brain is seen in lateral view. The longitudinal axes of the hemispheres diverge from the midline posteriorly by about  $30^\circ$ ; the anterior divergence of the optic lobes from the midline has increased to more than  $45^\circ$ . Figure 100, which shows dorsal views of the chick embryo's brain from the fourth to the twentieth day, may be consulted for better understanding of the rotation of the optic lobes. These structures also swing downward anteriorly and outward basally.

Experiments on the chick embryo (Huber, 1949a) indicate that the movement of the optic lobes is entirely passive, dependent upon the growth and displacement of the hemispheres dorsally and posteriorly; it does not take place when the evolution of the telencephalon is prevented by exposure to X-rays on the third or fourth day of incubation. According to Huber (1949b), the development of the face, and particularly of the lower beak, is the principal underlying cause of the shifting of various parts of the chick's brain between the sixth and the tenth day of incubation. During this period, the total length of the brain anterior to the metencephalon decreases to the same extent as, and therefore at the expense of, the diencephalon, although the telencephalon is growing longer at an almost constant rate. It thus appears that the hemispheres themselves are displaced toward the rear, presumably because of the growth of the structures of the face. Kupffer (1906) noted that the interorbital septum, in the chick of 7 days' incubation, rises to the level of the torus transversus. He ascribed the change in the position of the lamina terminalis to this fact. The relationship of the brain to the other cranial structures of the 8- to 9-day chick is apparent in Fig. 96-D, and comparison with Fig. 96-A, B, and C will reveal the changes which have taken place since the third day.

In Fig. 101 is presented a series of small, diagrammatic cross sections of the brain of the canary (*Serinus canaria*) embryo at a stage indicated by Boss (1913) as approximately the same as that shown in Fig. 95-C<sub>1</sub> and C<sub>2</sub>, but which appears to be somewhat more advanced. The first of these sections is taken through the hemispheres and the eyes, anterior to the rest of the brain, and the remaining sections are at successively more posterior levels; the last is through the myelencephalon, posterior to the cerebellum. These diagrams indicate very clearly the almost complete occlusion of all the ventricular spaces of the brain by the greatly increased thickness of the walls, which form the mass of brain substance.

A median longitudinal section through the lapwing (*Vanellus vanellus*) embryo's brain is shown in Fig. 99-C. This stage corresponds approximately to that found in the 9- to 10-day chick embryo.

Immediately apparent is the straightening of the axis of the brain. The



cranial flexure has virtually disappeared and the cervical flexure is much diminished.

The olfactory lobe has developed as a narrow anterior prolongation of the hemisphere. The telencephalon medium is so greatly shortened as to be practically nonexistent, and the anterior commissure,<sup>1</sup> at the site of the torus transversus, now occupies an anterior instead of a ventral or antero-ventral position. The chorioid plexus of the lateral ventricle, formerly anterior to the velum transversum, now lies lateral to it, and its folds are

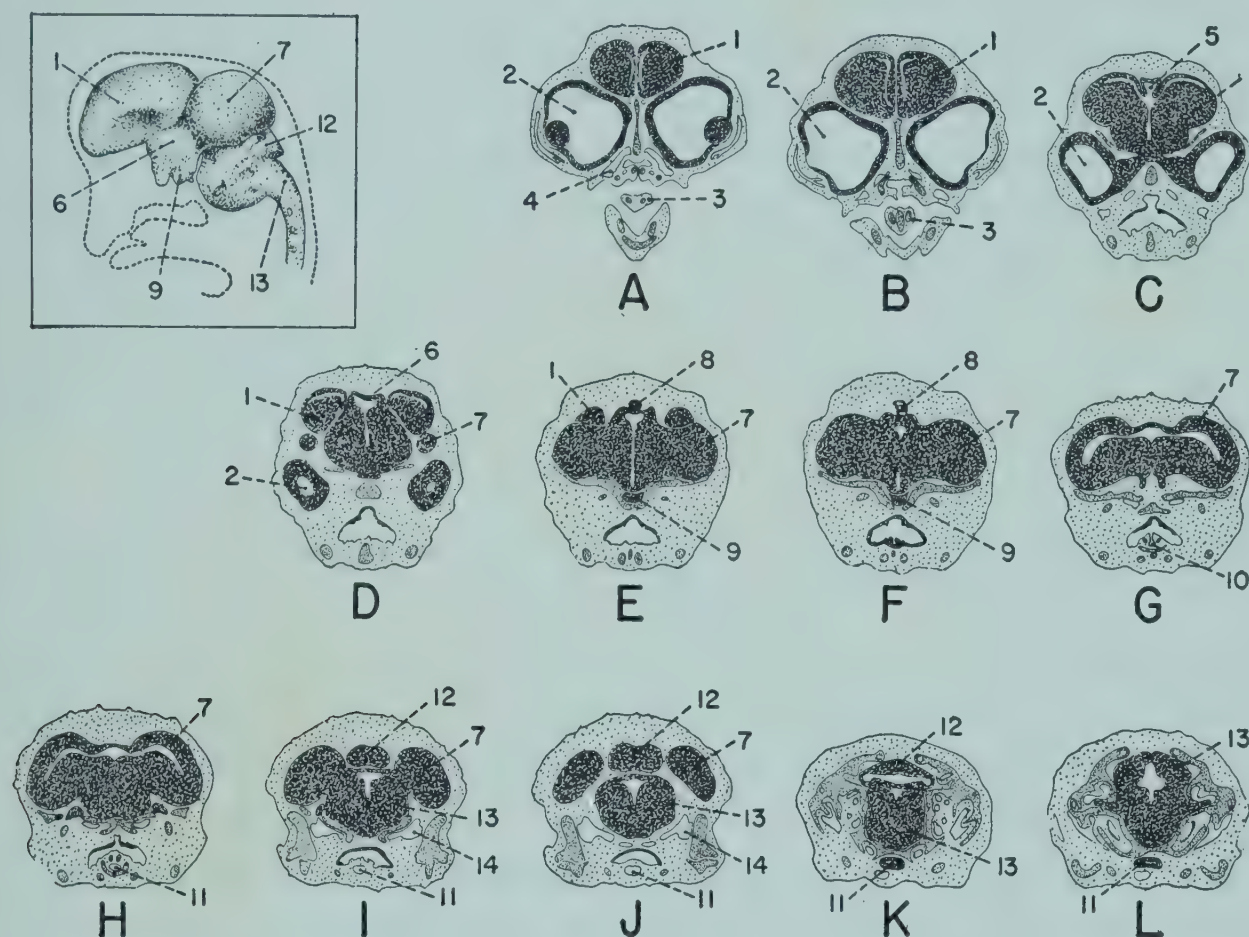


Fig. 101. A series of cross sections through the brain of the canary (*Serinus canaria*) embryo at a stage of development found in the chick embryo's brain at approximately 8 to 9 days of incubation. (Redrawn with modifications after Boss, 1913.)

From A to L the sections are at progressively more posterior levels. All cross sections  $\times 3$ .

1, hemisphere; 2, eye; 3, tongue; 4, sinus orbitalis; 5, parencephalon; 6, diencephalon; 7, optic lobe; 8, epiphysis; 9, hypophysis; 10, larynx; 11, trachea; 12, cerebellum; 13, rhombencephalon; 14, tympanic cavity.

no longer visible in sagittal section. The foramen of Monro is a mere slit. The lumen of the paraphysis communicates with it.

The parencephalon is narrow anteroposteriorly, and the inner surface of its roof is supplied with the chorioid plexus of the third ventricle. The epiphysis is large and prominent, and its lumen no longer communicates with the parencephalon. The habenular commissure has appeared close to the root of the epiphysis, in the roof of the parencephalon. The optic chiasma and the inferior commissure are greatly enlarged and the pars

<sup>1</sup> The anterior commissure connects the basal portions of the cerebral hemispheres.



optica is distinctly separated from the infundibulum, chiefly because of the elongation of the recessus infundibuli in the basal direction. The posterior opening from the infundibular cavity has been occluded, due to the great increase in the area of contact between the two sides of the brain. The infundibular commissure is now present in the posterior wall of the infundibulum.

As shown by Kuhlenbeck (1936), the development of the diencephalon during this period is characterized by the gradual disappearance of many of the sulci previously described. The system of longitudinal zones is obliterated by the further differentiation of the brain. The sulcus parencephalicus is still deep in the 9-day chick, but it is a short, longitudinal furrow. The sulcus intraencephalicus anterior and the sulcus lateralis infundibuli are much weaker than before, and only a trace remains of the sulcus diencephalicus ventralis. The sulcus diencephalicus dorsalis and the sulcus limitans, however, are clearly visible. The synencephalon has practically disappeared; according to Kuhlenbeck (1936), its caudal portion has become part of the mesencephalon, and its anterior section has merged with the parencephalon.

The optic lobes, as a result of their increase in size, have become demarcated by a furrow from the median portion of the mesencephalon, or lamina commissuralis. The lamina commissuralis has approached the basal portion of the mesencephalon as if it had collapsed downward, and the lumen of the median part of the mesencephalon is thereby transformed into a channel, narrow in the vertical dimension and continuous with the lumen of the isthmus. The aqueduct of Sylvius, connecting the third and fourth ventricles, has thus come into being. The lamina commissuralis contains the tectal commissure and the mesencephalic root of the trigeminal nerve (Tello, 1923). The commissura veli can be seen immediately posterior to the decussation of the trochlear nerve and anterior to the cerebellar commissure.

The pontine flexure has increased greatly and the cerebellar portion of the brain is bulbous and almost as prominent in the vertical direction as the other dorsally bulging vesicles. The medial extensions of the grooves separating the main body of the cerebellum from the laterally projecting anlagen of the flocculi have met in the midline, thus forming a continuous but shallow transverse furrow, the posterolateral fissure (Larsell, 1948). No other transverse fissures are present in the cerebellum of the 9-day chick embryo (Brouwer, 1913; Ingvar, 1918).

In the 9.5-day chick embryo (as in the 11-day duck, *Anas platyrhynchos*, embryo), however, there are three additional transverse furrows (Ingvar, 1918; Larsell, 1948), which, from anterior to posterior, are the fissura prima, the prepyramidal fissure, and the fissura secunda. The fissura prima is on the anterior surface of the cerebellum and is the boundary between the anterior



and posterior lobes of this part of the brain. The two halves of the corpus cerebelli have virtually completed their fusion in the midline. The lateral projections are thicker and enclose the lateral recesses of the fourth ventricle. Anterior and dorsoposterior views of the 11-day duck embryo's cerebellum may be seen in Fig. 103 and a diagrammatic midsagittal section in Fig. 104-A. A lateral view of the 9.5-day chick embryo's cerebellum is presented in Fig. 104—Insert *a*).

### *The Later Developmental Period*

Detailed studies of the avian brain during the late developmental period are not numerous. This stage, however, is characterized principally by the continued enlargement of the cerebellum and the cerebral hemispheres, the latter growing chiefly in the posterior direction.

A dorsal view of the chick's brain as it appears on the twelfth incubation day is given in Fig. 100-F. Comparison with Fig. 100-E reveals that both the hemispheres and the cerebellum are much larger in Fig. 100-F and that the optic lobes have continued to rotate until their long axes are perpendicular to the long axis of the brain. The optic lobes appear to be squeezed between the hemispheres and the cerebellum. The myelencephalon (medulla oblongata) has assumed its characteristic shape, and the median raphe traverses the length of its dorsal surface.

The lateral view and the cross sections of the 13-day chick embryo's brain shown in Fig. 102 are interesting chiefly because they show that the optic lobes are sinking to a lower position and that their longest diameter now runs in the dorsoventral direction.

On the next (fourteenth) day, the great increase in the size of the cerebellum is revealed in a dorsal view of the brain (Fig. 100-G). The hemispheres are also considerably larger, and they appear triangular when seen from above. The optic lobes are now virtually lateral structures. In a lateral view (cf. Fig. 102), the cerebellum is the part of the brain that is most prominent dorsally. Cross sections through the optic lobes (cf. Fig. 102) show that the cavities within the latter are being rapidly obliterated.

Two days later, on the sixteenth day, the cerebellum has grown forward to the level of the posterior poles of the hemispheres, as a dorsal view shows (cf. Fig. 100-H), and the hemispheres have started to overlap the dorsal surface of the optic lobes. A cross section (cf. Fig. 102) through the optic lobes indicates the forward and dorsal enlargement of the cerebellum, several transected folia of which appear in the midline between and above the optic lobes.

After the sixteenth day of incubation, the cerebellum continues to develop until, by the eighteenth day, it makes contact with the hemispheres, which have meantime further overgrown the optic lobes (cf. Fig. 100-I). As the result of pressure from both the cerebellum and the hemispheres, the optic



lobes have been pushed not only downward but also forward and ventrally, as shown in the lateral view of the brain given in Fig. 102. Sections through the optic lobes (cf. Fig. 102) show the lateral position of these structures as well as the increased number of cerebellar folia now present at this level. A section taken through the anterior portion of the optic lobes also includes the posterior part of the overlapping hemispheres and, in the upper mid-line, the epiphysis.

In Fig. 100-J and Fig. 102 are presented dorsal and lateral views, respectively, of the chick's brain on the twentieth day of incubation. These

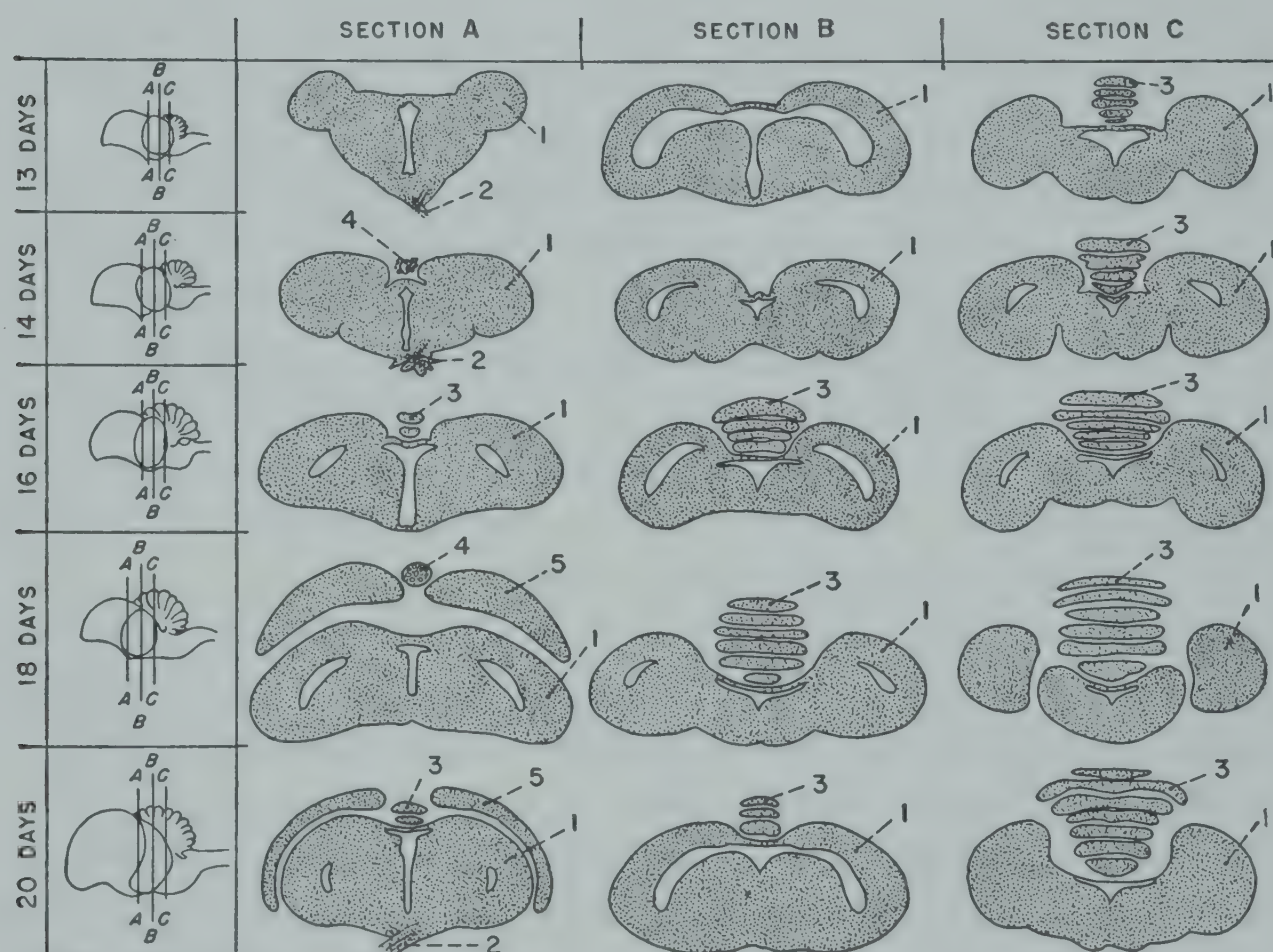


Fig. 102. The chick embryo's brain at various ages from 13 to 20 days' incubation, inclusive, shown in sections taken transversely across the optic lobes. All  $\times 2$ . (Redrawn with modifications after Oliver, 1912.)

*Inserts:* left lateral views of the brain, showing diagrammatically the levels at which the sections were taken.

1, optic lobe; 2, optic chiasma; 3, cerebellar convolution; 4, epiphysis; 5, hemisphere.

illustrations show that the growth of the hemispheres and the cerebellum has proceeded at a rapid rate. The optic lobes are now almost in a ventro-lateral position, since the hemispheres are overgrowing them in both the anteroposterior and dorsoventral directions and the cerebellum, above the optic lobes, is enlarging in the mediolateral direction. Sections through the optic lobes at this age may also be found in Fig. 102.

The development of the cerebellar fissures and folia during the latter half of the chick's and the duck's (*Anas platyrhynchos*) embryonic period has been studied by Ingvar (1918) and Larsell (1948). It will be recalled



that four transverse fissures—the posterolaterals, secunda, prepyramidalis, and prima—are present in the cerebellum of the 9.5-day chick and the 11-day duck embryo. In the duck, another fissure, called by Larsell the uvular sulcus 1, has appeared before the twelfth day caudal to the fissura secunda, and there is a suggestion of the posterior superior fissure between the fissura prima and the prepyramidal fissure.

In the 13-day duck (*Anas platyrhynchos*) embryo (approximately corresponding in development to the 11-day chick embryo), the fissurae secunda and prepyramidalis have deeply penetrated the substance of the cerebellum,

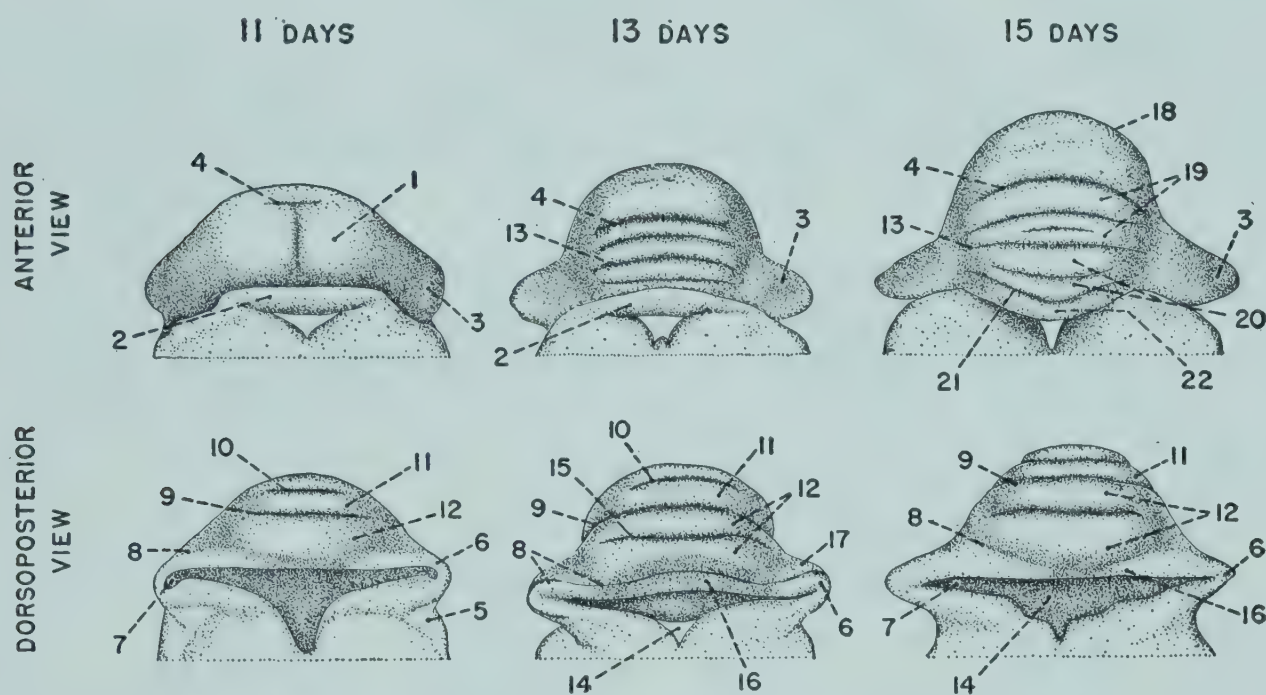


Fig. 103. The cerebellum of the domestic duck (*Anas platyrhynchos*) embryo at 11, 13, 15 days' incubation, shown in anterior and dorsoposterior aspect. All  $\times 4$ . (Redrawn with modifications after Larsell, 1948.)

1, anterior lobe of cerebellum; 2, anterior medullary velum; 3, auricle; 4, fissura prima; 5, medulla oblongata; 6, flocculus; 7, lateral recess of fourth ventricle; 8, posterolateral fissure; 9, fissura secunda; 10, prepyramidal fissure; 11, pyramis; 12, uvula; 13, preculminate fissure; 14, fourth ventricle; 15, uvular sulcus 1; 16, nodulus; 17, paraflocculus; 18, declive; 19, culmen; 20, central lobe; 21, precentral fissure; 22, lingula.

and the uvular sulcus 1 has extended laterally on each side almost to the base of the flocculi. A shallow furrow has appeared between the posterior superior fissure and the fissura prima, and the anterior lobe of the cerebellum (that is, the portion anterior to the fissura prima) is now divided into four main folia by three transverse fissures. This stage of development is indicated in Fig. 103; in Fig. 104-B, which shows a sagittal section and posterior view of the 13-day duck's (*Anas platyrhynchos*) cerebellum; and in Fig. 104—Insert *b*, representing a lateral view of the 11-day chick's cerebellum.

Further subdivision of the folia of the anterior lobe has occurred in the duck (*Anas platyrhynchos*) by the fifteenth day (cf. Fig. 103); and the six primary and secondary folia present in this lobe in the newly hatched duck



have formed by the twentieth day. On the eighteenth day, uvular sulcus 2 appears between the fissura secunda and uvular sulcus 1 (cf. Fig. 104-C). Two definite secondary folia are present at 18 days between the posterior superior fissure and the fissura prima; this number increases to three by the twentieth day (cf. Fig. 104-D). There are ten primary folia and seven secondary ones in the cerebellum of the newly hatched duck (cf. Fig. 104-E).

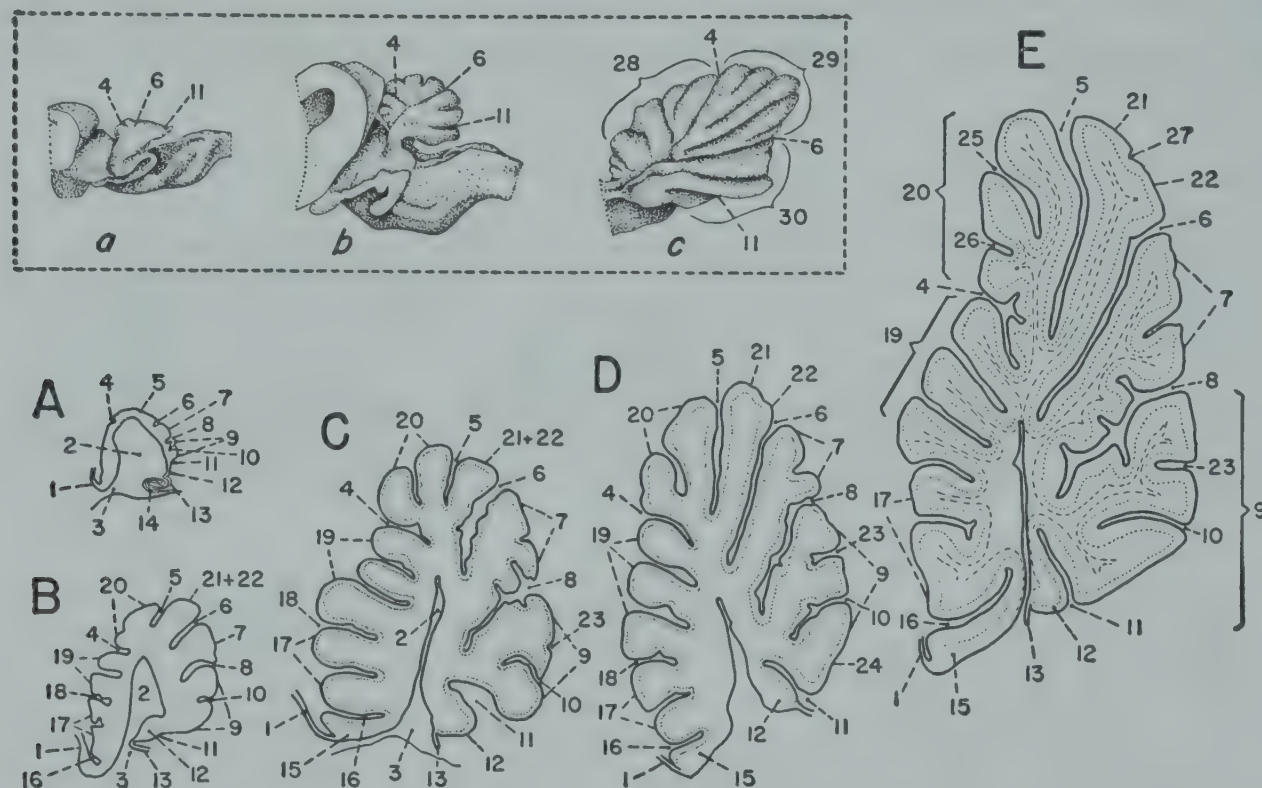


Fig. 104. The cerebellum of the duck (*Anas platyrhynchos*) embryo of various ages, shown in diagrammatic representations of midsagittal sections. (Redrawn with modifications A to E, after Larsell, 1948; and *a* to *c* after Ingvar, 1918.)

A, at 11.5 days; B, 13 days; C, 18 days; D, 20 days of incubation; E, hatching. All  $\times 6$ .

*Insert:* left lateral external views of the chick embryo's cerebellum at *a*, 9.5 days, *b*, 11 days, *c*, 16 days of incubation. All  $\times 3$ .

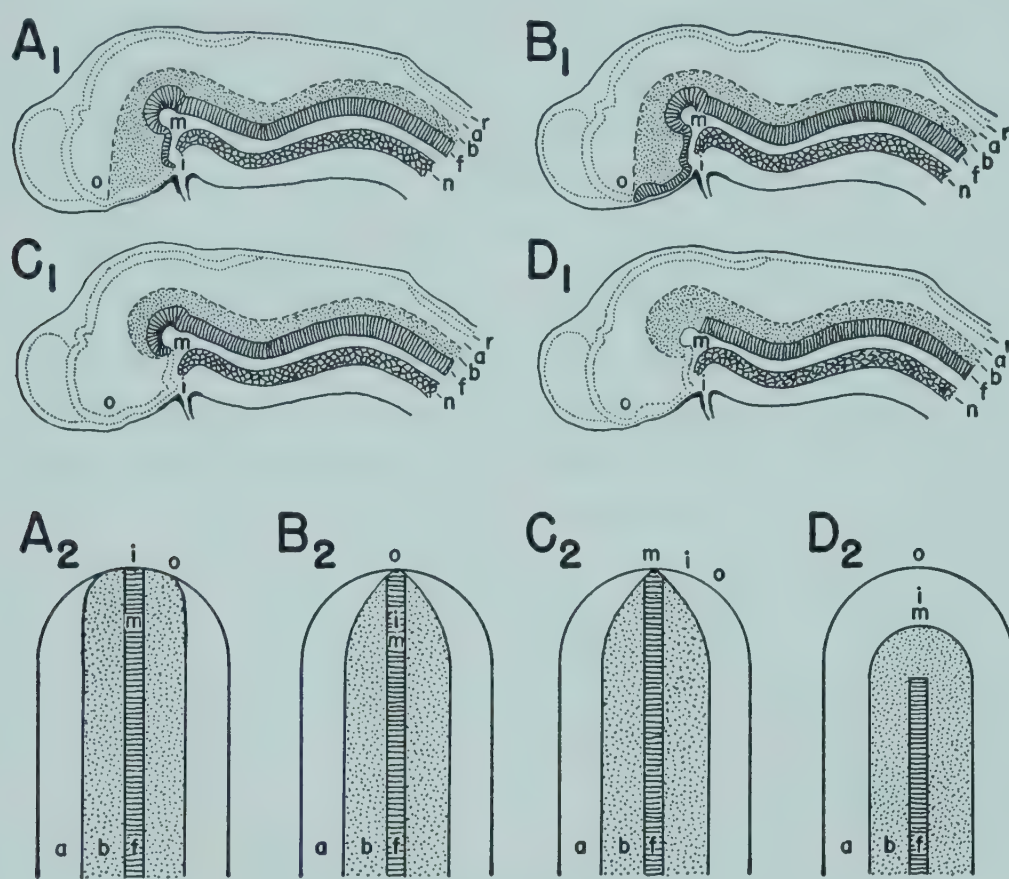
1, anterior medullary velum; 2, cerebellar ventricle; 3, fourth ventricle; 4, fissura prima; 5, posterior superior fissure; 6, prepyramidal fissure; 7, pyramis; 8, fissura secunda; 9, uvula; 10, uvular sulcus 1; 11, posterolateral fissure; 12, nodulus; 13, posterior medullary velum; 14, chorioid plexus; 15, lingula; 16, precentral fissure; 17, central lobule; 18, preculminate fissure; 19, culmen; 20, declive; 21, folium vermis; 22, tuber vermis; 23, uvular sulcus 2; 24, uvular sulcus 3; 25, declival sulcus 1; 26, declival sulcus 2; 27, horizontal fissure; 28, anterior lobe; 29, median lobe; 30, posterior lobe.

### The Plan of the Brain

The morphological divisions of the brain laid down during the early phases of development can be analyzed in relation to the organization of the central nervous system into longitudinal plates or laminae (*His*, 1888). As previously mentioned, these plates are the more or less nonnervous roof and floor plates, and, on either side, the lateral plate, which is in turn divided into the dorsal or alar (sensory) plate and the ventral or basal (motor) plate, separated from each other by the sulcus limitans.



His's original concept implied that all four of these zones extended to the anterior limit of the neural plate (Fig. 105-A<sub>2</sub>). The unclosed, outspread neural plate is divided into two halves by the future floor plate, which, to His, represented a previous line of suture resulting from concrescence (see Chapter 3). On either side of the floor plate lies the basal plate, flanked by the alar plate. With the closure of the neural tube, the dorsal line of suture joins the two alar plates, the anterior extremity of this line of suture marking the position of the future optic (preoptic) recess (Fig. 105-A<sub>1</sub>). The floor plate terminates at the infundibular recess, and an anterior line



**Fig. 105.** Diagrams representing the plan of the vertebrate brain as suggested by (A<sub>1</sub> and A<sub>2</sub>) His (1888), (B<sub>1</sub> and B<sub>2</sub>) Johnston (1909), (C<sub>1</sub> and C<sub>2</sub>) Schulte and Tilney (1915), and (D<sub>1</sub> and D<sub>2</sub>) Kingsbury (1922). The four longitudinal plates are shown on the flat medullary plate in A<sub>2</sub>, B<sub>2</sub>, C<sub>2</sub>, and D<sub>2</sub>, and their disposition and extent in the vesicular embryonic brain are indicated in A<sub>1</sub>, B<sub>1</sub>, C<sub>1</sub>, and D<sub>1</sub>.

r, roof plate; a, alar plate; b, basal plate; f, floor plate; n, notochord; m, mammillary recess; i, infundibulum; o, preoptic recess.

of suture extends from the infundibular recess to the optic recess and joins the two basal plates.

More recent workers have questioned this theory and have made several alternative suggestions. Johnston (1909) and Schulte and Tilney (1915) disagreed with His regarding the disposition of basal plate material in the neural plate. In their two schemata, the anterior boundary of the neural plate is formed solely of alar plate material, divided medially by the anterior end of the floor plate material (Fig. 105-B<sub>2</sub> and C<sub>2</sub>). Johnston (1909), however, extended the floor plate to the preoptic recess (Fig. 105-B<sub>1</sub>) and



pointed out that the infundibular recess of His actually develops into the postoptic recess. Schulte and Tilney (1915), on the other hand, terminated the floor plate at the mammillary recess, even farther caudally than the point designated by His. Consequently, the anterior suture of the neural tube was increased in length to include the entire region between the preoptic and mammillary recesses, contrary to the known facts of development. The significant feature of this latter plan is that the anterior end of the basal plate was placed at the mammillary recess (Fig. 105-C<sub>1</sub>).

A revised brain plan was proposed by Kingsbury (1920, 1922) on the basis of morphological evidence obtained from the study of chick embryos. Both the alar and basal plates are self-continuous around the anterior end of the floor plate (Fig. 105-D<sub>2</sub>), and the floor plate terminates in the brain at the caudal boundary of the mesencephalon, at the same level where the notochord ends anteriorly (Fig. 105-D<sub>1</sub>). This analysis accounts for the fact that sagittal sections of the brain reveal only the ependymal layer and neuroglia fibers in the floor of the rhombencephalon but show ependymal, mantle, and marginal layers in the floor of more anterior portions. Thus, anterior to the rhombencephalon, the primarily nervous material is continuous across the floor of the brain, and the floor has therefore the same morphological character as the lateral walls.

Kuhlenbeck (1935) analyzed the composition of the various segments of the brain as follows: the telencephalon and parencephalon consist of roof plate and alar plate; the synencephalon and mesencephalon contain roof plate, alar plate, and basal plate; and the rhombencephalon is composed of all four longitudinal laminae.

The coextensiveness of floor plate and notochord proposed by Kingsbury is consistent with the evidence presented by Wetzel (1932, 1936) that, from the moment regression of Hensen's node begins, the notochord is laid down by the deeper portion of the node and the floor of the neural tube by the more superficial portion (see Chapter 3).

### Cytoarchitectural Development of the Chick Embryo's Brain

The cytoarchitectural development of the brain consists of the grouping of functionally similar cell bodies into centers or nuclei and the aggregation of fibers to form the great connecting systems and tracts. This aspect of brain development begins at a relatively early age and proceeds rapidly, so that the essential microscopic structure of the brain is recognizable well before the end of the incubation period.

Neurological studies of the cytoarchitecture of the embryonic avian brain are not numerous, and the following account is therefore incomplete. Furthermore, it is mainly descriptive; readers who wish to determine the functional significance of the structures mentioned may consult neurological texts. For the weight of the embryonic brain see Appendix, Table V.



*The Early Development of Fiber Tracts*

The appearance of fiber tracts in the avian brain precedes the development of the higher correlation centers. Neurofibrillar differentiation begins in the brain of the chick during the latter half of the second day of incubation, when the embryo has not less than 15 somites. Bundles of fascicles of individual fibers gradually form the primordia of the great nerve pathways found in the adult brain.

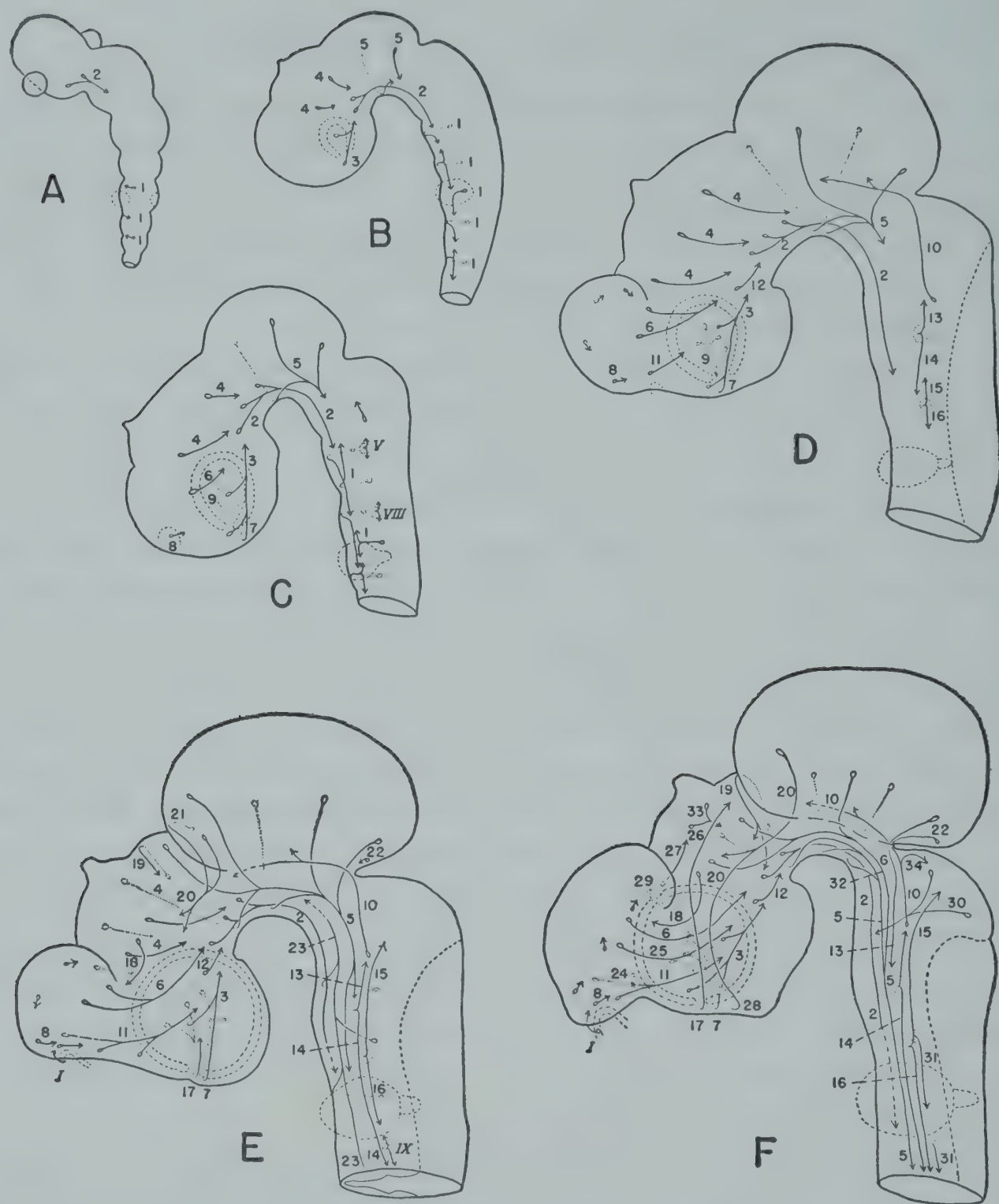
The earliest recognizable fiber tracts are concerned with simple direct reflex mechanisms, although descending fibers are relatively well developed before the first local reflex arcs are present. Primitive integrating mechanisms (rhombencephalic elements of the reticulo-spinal tract and the diencephalic components of the medial longitudinal fasciculus) appear before the formation of primary motor neurones, thus reversing the order of development found in mammals (*Windle and Austin, 1936*).

*From the 15- to the 37-somite stage.* The first fibers to develop in the brain have been found in the rhombencephalon near the otic placode and in either the midbrain or the region of the diencephalo-mesencephalic junction (*Cowdry, 1914; Tello, 1923; Windle and Austin, 1936*) of the 15- to 18-somite chick embryo. Differentiating (primary bipolar) neuroblasts appear first in the lateral wall of the rhombencephalon and send their axons toward the floor. Most of the rhombencephalic neuroblasts probably become neurones of the reticular formation (*Windle and Austin, 1936*). A few cells in the rostral end of the mesencephalon represent the rostral component of the medial longitudinal fasciculus, which will occupy the medial part of the basal plate.

At 20 somites (Fig. 106-A), axons of the rhombencephalic neuroblasts may cross the floor plate to the opposite side. The medial longitudinal fasciculus consists of a small bundle of fibers proceeding no farther caudally than the anterior end of the notochord. At 22 somites, however, it has extended to the caudal part of the cranial flexure. At this stage, rhombencephalic neuroblasts destined to become neurones of the medullary reticular formation are present from the level of the trigeminal nerve to the anterior end of the spinal cord. In the vicinity of the otocyst, some of the axons which cross the floor, as well as some which do not, turn caudally and form a discontinuous longitudinal tract (*Windle and Austin, 1936*). Vagal motor neuroblasts have appeared and visceral motor roots of the vagus (X cranial) nerve are in the very early stages of formation.

The diagram presented as Fig. 106-B gives an indication of the development of the fiber tracts in the 27-somite chick embryo. It can be seen that there is a system of relatively short, ventrolateral, descending fibers making up a discontinuous longitudinal pathway, the medial longitudinal fasciculus, which now extends to the level of the trigeminal nerve. Newly formed





**Fig. 106.** The progressive development of fiber tracts in the brain of the chick embryo, shown diagrammatically. (Redrawn with modifications after Windle and Austin, 1936.)

A, at 43 hours (20 somites); B, at 48 hours (27 somites); C, at 60 hours (32 somites); D, at 73 hours (37 somites); E, at 4 days; F, at 5 days.

1, reticular neurones of rhombencephalon; 2, medial longitudinal fasciculus; 3, hypothalamo-subthalamic tracts; 4, thalamo-tegmental tract; 5, tecto-bulbar tracts; 6, striato-subthalamic tract; 7, dorsal supraoptic decussation; 8, olfactory neurones; 9, retinal neurones; 10, trigeminal lemniscus; 11, olfacto-hypothalamic tract; 12, mammillo-tegmental tract; 13, ascending trigeminal primary tract; 14, descending trigeminal primary tract; 15, primary vestibulo-cerebellar tract; 16, descending vestibular primary tract; 17, optic chiasma; 18, thalamo-striatal tract; 19, posterior commissure; 20, tecto-thalamic tracts; 21, tectal commissure; 22, trigeminal mesencephalic neurones; 23, bulbo-mesencephalic fibers (medial lemniscus); 24, anterior commissure; 25, olfacto-subthalamic tracts; 26, optic tract; 27, stria medullaris; 28, ventral supraoptic decussation; 29, parolfacto-habenular tract; 30, cerebello-bulbar tract; 31, tractus solitarius; 32, mesencephalo-trigeminal tract; 33, habenulo-peduncular tract; 34, trigemino-cerebellar tract; I, V, VIII, IX, cranial nerves.



fibers originating dorsal and ventral to the optic stalk unite caudal to it and constitute a hypothalamo-subthalamic tract. Neuroblasts in the lateral wall of the diencephalon send axons ventrally, initiating the formation of thalamo-tegmental or thalamo-bulbar tracts. Future tecto-bulbar and tecto-spinal tracts are represented by fibers proceeding downward on the lateral wall of the mesencephalon to join the medial longitudinal fasciculus of the same side or to cross in the floor of the mesencephalon and descend on the opposite side (*Tello, 1923; Windle and Austin, 1936*). The number of rhombencephalic reticular neuroblasts has increased, especially in the otic region. Most of their axons cross the floor and turn caudally, although a few turn rostrally. Most cells in the rhombencephalon contribute to the reticulospinal pathways or to the neuropile (unmyelinated fiber mesh) of the medullary reticular formation (*Windle and Austin, 1936*).

In the 32-somite chick embryo (Fig. 106-C), descending axons representing early striato-subthalamic elements appear at the caudal border of the telencephalon. They proceed only a short distance (*Tello, 1923; Windle and Austin, 1936*). A few neuroblasts with short axons are present in the olfactory region and give rise to the olfacto-hypothalamic or olfacto-subthalamic tracts. Fibrillogenesis is starting in the retina at this stage (*Windle and Austin, 1936*), if not earlier (*Tello, 1923*). Some of the fibers of the hypothalamo-subthalamic system now cross to the opposite side, forming a small commissure ventral to the optic stalk. This is the primordial dorsal supraoptic decussation. At the level of the oculomotor nucleus there has appeared a small ventral commissure, where some of the fibers of the tecto-spinal system cross. A lateral longitudinal fascicle, with fibers directed anteriorly, is beginning to form in the rostral part of the rhombencephalon; this is the anlage of the trigeminal lemniscus (*Windle and Austin, 1936*).

Comparison of Fig. 106-C and D will show the progress in fibrillogenesis which has occurred by the 37-somite stage. It can be seen that all of the fiber systems just described (except the reticular neurones of the rhombencephalon, which have been omitted from the diagram) are considerably better developed than in the 27-somite embryo of Fig. 106-B. In addition, a few new elements are apparent. Neuroblasts with very short axons are present in the rostromedial and dorsomedial portions of the hemisphere, but not in the lateral wall (*Windle and Austin, 1936*). The axons of the optic nerve now enter the optic stalk but do not reach the midline. Along the dorsal border of the mammillary recess there is a short mammillo-tegmental fascicle which is incorporated at its caudal end into the medial longitudinal fasciculus (*Tello, 1923; Windle and Austin, 1936*). Throughout the rhombencephalon both ascending and descending pathways are present, though not shown in Fig. 106-D. The ventral commissure is conspicuous and is formed chiefly of fibers passing into the ventral longi-



tudinal fasciculus. Ascending and descending fibers of the trigeminal nerve and of the vestibular nerve also appear in Fig. 106-D.

*At 4 days.* The increasing complexity of the fiber tracts is apparent in Fig. 106-E, which depicts the stage of fiber development attained in the 4-day chick embryo's brain. Fibers of the olfactory nerve are beginning to form (*Tello, 1923; Windle and Austin, 1936*), and the olfacto-hypothalamic tract reaches as far as the nucleus of the hypothalamo-subthalamic tract. The optic chiasma has developed (*Tello, 1923*), although the fibers from the retina are not as yet very long (*Windle and Austin, 1936*). The anterior commissure, not visible in Fig. 106-E, is also present, though small. The first elements of the thalamo-striatal tract, directed rostrally, are seen in the lateral wall of the diencephalon. Other ascending (tectothalamic) fibers pass from the rostral part of the midbrain into the diencephalon. The posterior commissure has been formed immediately anterior to and partly within the optic tectum; according to *Tello (1923)*, this commissure is visible at 36 somites. Additional fibers caudal to the posterior commissure make up the tectal commissure. Trigeminal mesencephalic neuroblasts extend a short distance forward from the isthmus on to the tectal wall. The fibers of the trigeminal lemniscus now extend as far anteriorly as the diencephalon. The ascending fibers of the vestibular nerve veer dorsally into the cerebellar anlage as the early vestibulo-cerebellar tract. In the rhombencephalon, ascending fibers ventral and lateral to the medial longitudinal fasciculus form the medial lemniscus (bulbo-mesencephalic tract). The oldest neurofibrillar structures of the rhombencephalon are building the ascending and descending components of the medial longitudinal fasciculus and reticulo-spinal tracts of the same and opposite sides (*Windle and Austin, 1936*).

*At 5 days.* In spite of the great complexity of the neurofibrillar structures in the 5-day chick embryo's brain, relatively few of the adult pathways can be identified (*Windle and Austin, 1936*). Figure 106-F is given to clarify the following description of new developments since the fourth day.

Many neurones of the olfactory system are present in the telencephalon. The olfacto-hypothalamic tract, arising in the medial wall of the hemisphere, now ends in the wall of the optic recess among neurones of the hypothalamo-subthalamic tract. The olfacto-subthalamic tract originates in the lateral wall of the hemisphere at a more dorsal level and ends in the subthalamus or ventral thalamus (*Windle and Austin, 1936*).

Fibers from the corpus striatum also end in the ventral thalamus. A few axons arising along the dorsal border of the corpus striatum are directed toward the cortex of the hemisphere (*Windle and Austin, 1936*).

The optic chiasma is large and the fibers of the optic tract course dorsally over the surface of the diencephalic wall, passing over the primordial nucleus rotundus (which gives rise to ascending fibers of the thalamo-



striatal tract passing to the corpus striatum). At this time, the optic tract disappears just anterior to the optic tectum (*Windle and Austin, 1936*), although some fibers may reach the latter (*Tello, 1923*).

In the anterior dorsolateral wall of the diencephalon, beneath the projecting hemisphere, a nucleus gives rise to the stria medullaris, which proceeds for a short distance caudally toward the habenular nucleus (*Windle and Austin, 1936*). The latter nucleus begins to form in the dorsolateral wall of the diencephalon on the fourth (*Tello, 1923*) or fifth day (*Windle and Austin, 1936*). It sends a few short fibers ventrally.

In the tegmentum, ascending fibers from the mesencephalon and rhombencephalon are mingled with crossed and uncrossed fibers of the thalamo-tegmental and thalamo-bulbar tracts from the lateral wall of the diencephalon (*Windle and Austin, 1936*).

The tecto-thalamic tracts have undergone considerable growth. One of these, the direct tract, proceeds from the rostral portion of the optic tectum as far as the nucleus rotundus. Its fibers are superficial to the descending fibers of the thalamo-tegmental tracts. From a similar point of origin, another tecto-thalamic tract courses over the thalamus to the optic recess, passing parallel and ventral to the optic tract. The fibers of this tecto-thalamic tract form the ventral supraoptic commissure as they cross to the opposite side of the brain, where they course rostrally almost to the thalamo-striatal tract or possibly as far as the nucleus rotundus (*Windle and Austin, 1936*).

The crossed tecto-spinal and uncrossed tecto-bulbar tracts, which contain most of the fibers from the optic tectum, have extended themselves farther caudally and may even reach the spinal cord. The crossed tecto-spinal tract lies ventral to the medial longitudinal fasciculus. The uncrossed tecto-bulbar tract lies lateral to it in regions rostral to the rhombencephalon. The mesencephalic tract of the trigeminal nerve, together with other fibers from the mesencephalon, reaches as far as the motor nucleus of the trigeminal nerve.

Cerebellar connections are starting to develop. A cerebello-bulbar tract is beginning to form, and a few fibers enter the rostral part of the cerebellar primordium from the trigeminal lemniscus (*Windle and Austin, 1936*).

Table 4 shows the approximate time of appearance (in incubation hours) of the various tracts in the early chick embryo's brain, as given by both *Windle and Austin (1936)* and *Tello (1923)*.

**The growth of longitudinal fiber tracts.** It has been pointed out that neurofibrillar differentiation in the brain of the chick starts in the rhombencephalon and at the diencephalo-mesencephalic border. Experimental evidence seems to indicate that the development of fiber tracts proceeds both rostrad and caudad from these two regions and that the meeting of ascending and descending fibers contributes to the elongation of the tracts



TABLE 4  
Fiber Tracts of the Early Chick Embryo's Brain  
in the Order of Their Appearance

Name of Tract	Fibers: Direct or Crossed	Time of Appearance (hours)	
		Windle and Austin (1936)	Tello (1923)
Reticulo-spinal (intersegmental neurones)	Both	38-40	40-42
Medial longitudinal fasciculus	Direct	40	40-42
Hypothalamo-subthalamic	Direct	Before 48	52-55
Tecto-bulbar and/or -spinal	Direct	Before 48	52-55
Spino-bulbar (intersegmental neurones)	Both (?)	48	46-48 (?)
Thalamo-tegmental or -bulbar	Direct	48	96
Tecto-bulbar and/or -spinal	Crossed	48	72
Optic tract (ganglion cells or retina)	Crossed	After 48	46-48
Striato-subthalamic	Direct	60	52-55
Olfacto-subthalamic	Direct	60	52-55
Olfacto-hypothalamic	Direct	60	52-55
Dorsal supraoptic decussation (hypothalamo-subthalamic)	Crossed	60	52-55
Primary trigeminal tract	Direct	60	52-55
Trigeminal lemniscus	Direct	60	
Primary vestibular (includes cerebellar root)	Direct	60	
Medial lemniscus	Both	73 (?)	72
Mammillo-tegmental	Direct	73 (?)	72
Tractus solitarius	Direct	After 73	72
Posterior commissure	Crossed	After 73	96
Striato-thalamic	Direct	96 (?)	
Thalamo-tegmental or -bulbar	Crossed	96	96
Tecto-trigeminal (mesencephalo-trigeminal)	Direct	96	
Tecto-thalamic	Direct	96	
Thalamo-striatal	Direct	96	
Anterior commissure	Crossed	96	
Olfactory tracts	Direct	96 (?)	
Olfactory nerve	Direct	96	96
Dorsal spinal funiculus	Direct	96	96
Tecto-thalamic (ventral supra- optic commissure)	Crossed	Before 120	
Stria medullares	Direct	96	96
Habenulo-peduncular	Direct	120	96
Habenular commissure	Crossed	120 (?)	
Trigemino- and vestibulo- cerebellar (secondary)	Direct	120	
Striato-cortical	Direct	120	



(*Rhines and Windle, 1944*). This observation, if correct, contradicts the theory of progressive cephalo-caudal neurofibrillar development which forms so important a part of the concept of stimulogenous fibrillation. It has been found that the experimental excision of various portions of the chick embryo's brain prior to the appearance of longitudinal tracts does not prevent the differentiation of cranial motor nuclei (*Rhines and Windle, 1944*), although Bok (1915) has predicted the formation of these nuclei upon the existence of electrical stimuli produced by the descent of the median longitudinal fasciculus. The manner in which fibers in the truncated brain follow the contour of the supporting framework tends to confirm the mechanical theory of nerve growth during embryonic development, rather than the theory of neurobiotaxis (*Rhines and Windle, 1944*).

Experimental data also strongly suggest that the rapid elongation of nerve fibers in the brain is only apparent, since the early tracts seem to be composed of a series of overlapping short fibers whose growth is relatively slow. The individual diencephalic fibers of the medial longitudinal fasciculus, for example, have progressed only as far as the metencephalon by the chick's sixth incubation day (4 days after their initial appearance), but the entire fasciculus extends as far as the lower levels of the rhombencephalon at that time (*Rhines, 1943*).

### *The Telencephalon*

The bird's telencephalon consists of two short, broad hemispheres. Their size is due principally to the great development of the somatic corpora striata which fill their interior. The olfactory bulbs are very small and the hippocampal formation is weakly developed. The posterior poles are formed by the amygdalar complex and extend caudally over the thalamus and the tectum. A small anterior commissure connects the two amygdalar regions. The lateral ventricles, which are small, are displaced dorsally and enclose a small chorioid plexus. There is no corpus callosum and the fornix system is vestigial. The ventral surface of the telencephalon is concave where it lies over the very large orbits.

The well-developed corpus striatum is a sensory-motor correlation center. Its afferent connections are with the optic tecta and with the thalamus, and its efferent connections are with the cerebellum and the nuclei dealing with ocular and masticator musculature.

The avian peculiarities of the chick's telencephalon appear between the seventh and the tenth day of incubation. The most detailed account of the development of the hemispheres in the chick is that of Kuhlbeck (1938).

**At 3 days.** The cytoarchitectural development of the chick's telencephalon starts during the third day of incubation (*Kuhlbeck, 1938*). It will be recalled that several longitudinal zones are apparent in the telencephalon of the chick embryo of about 40 somites, a stage reached in the



latter part of the third day of incubation. The wall of each hemisphere consists of a very thin, noncellular outer (marginal) layer and an internal, cellular (ependymal or germinal) layer, best developed in the basal region. There is also a thin, cellular mantle layer between the ependymal and marginal layers. The upper (pallial) and basal portions of the telencephalon are each divided into three zones, separated by very shallow limiting sulci. For convenience, the pallial zones, from dorsal to ventral, may be designated as D3, D2, and D1, and the basal zones, in the same direction, as B1, B2, and B3 (cf. Fig. 94). The zone B3 is present only in the fully evaginated portion of the hemisphere.

*At 5 to 6 days.* In the 120-hour chick, the telencephalic wall has increased noticeably in thickness in the basal zones, due chiefly to the further development of the mantle layer. In the pallial region, the mantle layer gradually diminishes in thickness through zones D1 and D2, and is almost nonexistent in zone D3 (cf. Fig. 94-G). In the fully evaginated portion of each hemisphere, the basal zone B3 is now on the medial wall, and a new zone, B4, has differentiated dorsal to it (this zone does not appear in Fig. 94-G). In the more caudal regions, a cell-free lamina or zona limitans appears in the mantle layer approximately opposite the sulcus which, on the internal surface of the ventricle, lies between zones D1 and B1. This sulcus is the neo-palaeostriatal groove. The zona limitans will become the lamina medullaris dorsalis of the adult corpus striatum, separating the neostriatum, or nucleus epibasalis centralis (Kuhlenbeck, 1938), from the palaeostriatum augmentatum (mesostriatum), or nucleus basalis (Kuhlenbeck, 1938).

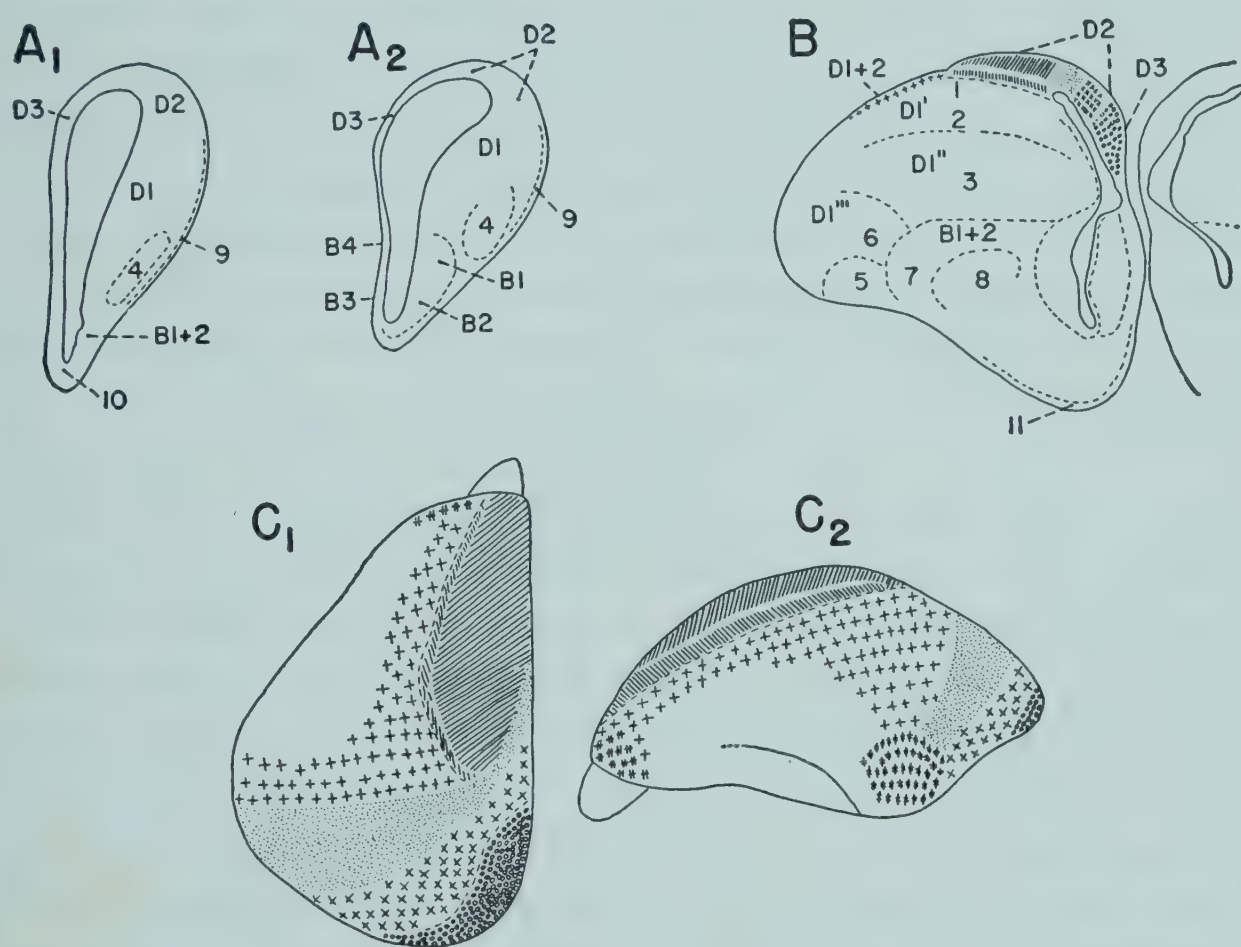
In the chick embryo of 5 days 16 hours of incubation, the telencephalic wall is much thicker in the zones D1 and B1, where the mantle and ependymal layers merge together diffusely (cf. Fig. 94-H). In the remainder of the pallial region these two layers are separated by a very narrow, lightly staining zone. The zona limitans has extended forward into the fully evaginated portion of the hemisphere.

*At 7 to 8 days.* At 7 days, the chick's telencephalon, hitherto resembling that of *Anamnia* in cross section, begins to take on a generalized sauropsidan pattern. In zones D1, B1, and B2, the mantle layer is considerably thicker than it was on the sixth day (cf. Fig. 94-I). Durward (1934) attributed this increase in mass in the brain of the sparrow (*Passer domesticus*) embryo of a comparable developmental stage not to migration or extension of any already existing cortical sheet, but to cell proliferation *in situ*. On the lateral surface of zones D1 and D2, a thin corticoid lamina has developed. In the more basal portion of D1, the primordium of the neostriatum (nucleus epibasalis centralis) has appeared. Lateral to this cell group is a denser mass which merges with the basal end of the corticoid lamina. Fibers of the lamina medullaris dorsalis are present in the zona limitans.



Cross sections through the rostral part of the right hemisphere, one taken at the level of the olfactory bulb and the other immediately posterior to it, are represented diagrammatically in Fig. 107- $A_1$  and  $A_2$ , respectively. At both these levels, the zone D1 extends much farther basalward than it does caudally, and zones B1 and B2 are decreased in size and are fused. In the lateral basal part of D1 may be seen the primordium of the nucleus epibasalis ventrolateralis (*Kuhlenbeck, 1938*).

On the eighth incubation day, cross sections of the chick's telencephalon show that the avian pattern is beginning to emerge. The corpus striatum is much more massive. The lateral portion of zone D2 adjoining zone D1 has



**Fig. 107.** The development of the cortex telencephali in the chick embryo. (Redrawn with modifications after *Kuhlenbeck, 1938*.)

$A_1$  and  $A_2$ , cross sections through the rostral part of the right hemisphere, at the level of the olfactory bulb and immediately caudal to the olfactory bulb, respectively ( $\times 10$ ); B, a cross section through the left hemisphere of the 10-day embryo ( $\times 10$ );  $C_1$  and  $C_2$ , the pallial areas projected on the surface of the left hemisphere of the 19-day embryo, dorsal view and lateral view from the left side, respectively ( $\times 4$ ).

$A_1$ ,  $A_2$ , and B show the further development of the basal zones B1, B2, B3, and B4 and the pallial zones D1, D2, and D3, seen also in Fig. 94.

1, dorsal hyperstriatum; 2, ventral hyperstriatum; 3, neostriatum; 4, nucleus epibasalis ventrolateralis; 5, archistriatum; 6, ectostriatum; 7, palaeostriatum augmentatum (mesostriatum); 8, palaeostriatum primitivum; 9, lateral corticoid area; 10, olfactory bulb; 11, tuberculum olfactorium (area ventralis anterior).

Oblique lines represent hyperstriatum accessorium; vertical lines, nucleus diffusus dorsolateralis; crosses, lateral corticoid area; crosses with two vertical or two horizontal bars, lateral cortical fields; x's and stippling, parahippocampal formations; circles, hippocampal formation.



thickened considerably and is sharply demarcated on the ventricular surface from zone D1 and from the thin, more medial portion of zone D2 adjoining zone D3. This thin medial segment of D2 comprises the primordium of the parahippocampal cortical fields. At this time it consists of a homogeneous deeper portion, continuous medially with D3, and of a superficial condensation of cells in its lateral half. This superficial formation is continuous with the lateral corticoid layer formed over D1 and D2 on the seventh day. According to Kuhlenbeck (1938), the basal end of the corticoid lamina over D1 and D2 represents, in rostral regions, the area praepyriformis of the adult brain.

**At 10 days.** By the tenth day, the lateral ventricle has become extremely narrow, and the progressive increase in the thickness of the telencephalic wall is most pronounced in zone D1 (Fig. 107-B). Within this zone can be seen, in the following order from above basalward, the superior and inferior dorsal epibasal nuclei (or dorsal and ventral hyperstriata), the nucleus epibasalis centralis (or neostriatum), the nucleus epibasalis centralis accessorius (or ectostriatum), and the nucleus epibasalis caudalis (or archistriatum). The last two nuclei are lateral to the basal regions B1 and B2, in which appear the nucleus basalis (palaeostriatum augmentatum, or mesostriatum) and the nucleus entopeduncularis anterior (palaeostriatum primitivum). The thickened lateral portion of zone D2 now contains the primordium of the nucleus diffusus dorsalis (hyperstriatum accessorium), a noncortical structure which largely replaces the dorsal plate of the cortex of reptiles (Craigie, 1936). Immediately basal to it lies the primordial nucleus diffusus dorsolateralis, continuous with the lateral corticoid layer. The nucleus diffusus dorsalis is continuous medially with the primordia of the parahippocampal cortical fields in the medial thinner part of zone D2. The primordia of the hippocampal cortical fields are apparent in zone D3, which is now situated in the dorsal portion of the medial wall of the hemisphere. The differentiation of a rudimentary tuberculum olfactorium (area ventralis anterior) may be seen over zones B1, B2, and B3 caudal to the olfactory bulb. More caudally, the medial portion of this basal cortex increases in distinctness. Zones B1 and B2 fuse near the foramen of Monro and gradually disappear more posteriorly. Cells belonging to these zones reach the surface and represent the caudal portion of the basal cortex, the area ventrolateralis posterior (Kuhlenbeck, 1938).

**At 11 days.** Very little change from the 10-day condition is apparent at 11 days, except for the increased distinctness of the nuclei. The nucleus diffusus dorsalis and the nucleus diffusus dorsolateralis extend to the enlarged rostral pole of the hemisphere. The anterobasal portion of the lateral corticoid lamina (the area praepyriformis) now bends around the rostral pole of the hemisphere and lies on both its medial and its lateral surface.

**At 12 to 19 days.** At 12 days, all the cell masses of the adult hemisphere



are present. During the remainder of the incubation period they grow and gradually attain their definitive appearance.

Figures 107-C<sub>1</sub> and C<sub>2</sub> show the various pallial cortical areas (or their homologues) projected on the surface of the left hemisphere of the 19-day chick embryo. The diagrams show both dorsal and lateral aspects. The nuclei diffusi (dorsalis and dorsolateralis), flanked laterally by the narrow corticoid layer, occupy the dorsomedial surface of the anterior two thirds of the hemisphere. The lateral corticoid layer has the same anteroposterior extent as the nuclei diffusi. In its posterior half, however, it widens and spreads halfway down the lateral surface of the hemisphere. At the most lateral point of its wide posterior portion, it is transformed into a true cortical plate and represents the lateral cortex. At its anterior end, it becomes cortical in the prepyriform area dorsal to the olfactory bulb. The parahippocampal and hippocampal cortical fields occupy the dorsolateral surface of the posterior one third of the hemisphere. In the anterior two thirds of the hemisphere these fields are, for the most part, restricted to the medial surface. Sections show that they have a laminated structure, although this feature is less distinct rostrally than elsewhere.

### *The Diencephalon*

The avian diencephalon may be divided into four major parts: the epithalamus, the dorsal thalamus, the ventral thalamus, and the hypothalamus. The first and last of these are least variable among vertebrates. The epithalamic centers in the bird deal with olfacto-somatic correlation and reflect the degree of development of the olfactory sensibility. The dorsal thalamus is large, reflecting the development of the sensory systems and the striatum. The ventral thalamus is an efferent center receiving impulses from the striatum and discharging on lower motor centers. Its development in birds also reflects the development of the striatum. The hypothalamus is concerned with visceral function and, in birds, shows many early mammalian features.

The development of cell masses in the avian diencephalon has been the object of special study by Kuhlenbeck (1937). This region of the brain passes through a period of organization into four longitudinal cell columns (Kuhlenbeck, 1936) which are broken up into many separate centers during the further course of development.

*At 3 to 4 days.* The first indication of the longitudinal zonal system can be detected in the chick embryo's diencephalon toward the end of the third day of incubation. The diencephalic wall consists of a thin marginal layer overlying a thicker cellular matrix. Early in the fourth day, in all regions except the epithalamus and the ventral anterior portion of the hypothalamus, the cellular matrix differentiates into a dense periventricular endymal (or germinal) layer and a thin, loose mantle layer. These layers



become progressively more distinct from rostral to caudal levels. In the mantle layer, a cell-free lamina (*zona limitans intrathalamica*) is present between the dorsal and ventral thalami.

*At 5 to 6 days.* In the 120-hour chick, the limits of the relatively undifferentiated cell columns coincide closely with the diencephalic sulci previously described. The epithalamus, however, reaches no farther caudally than the rostral limit of the posterior commissure. Posterior to the epithalamus there is a dorsalward extension of the dorsal thalamus which extends to the caudal end of the posterior commissure and within which will develop certain pretectal nuclei (*Kuhlenbeck, 1939*). The main portion of the dorsal thalamus is bounded posteriorly by the primordia of the mesencephalic tectal formation and the torus semicircularis, which are dorsal and ventral, respectively, to the sulcus lateralis mesencephali. The anterior end of the mesencephalic tegmentum forms the caudal boundary of the ventral thalamus and the hypothalamus. Figure 108-A is a diagram showing the cellular zones of the diencephalon projected upon the ventricular surface. (Fig. 108 should be compared with Fig. 97.)

The differentiation of mantle and ependymal layers is now more pronounced. In the epithalamus these layers are very thin (*Kuhlenbeck, 1939*).

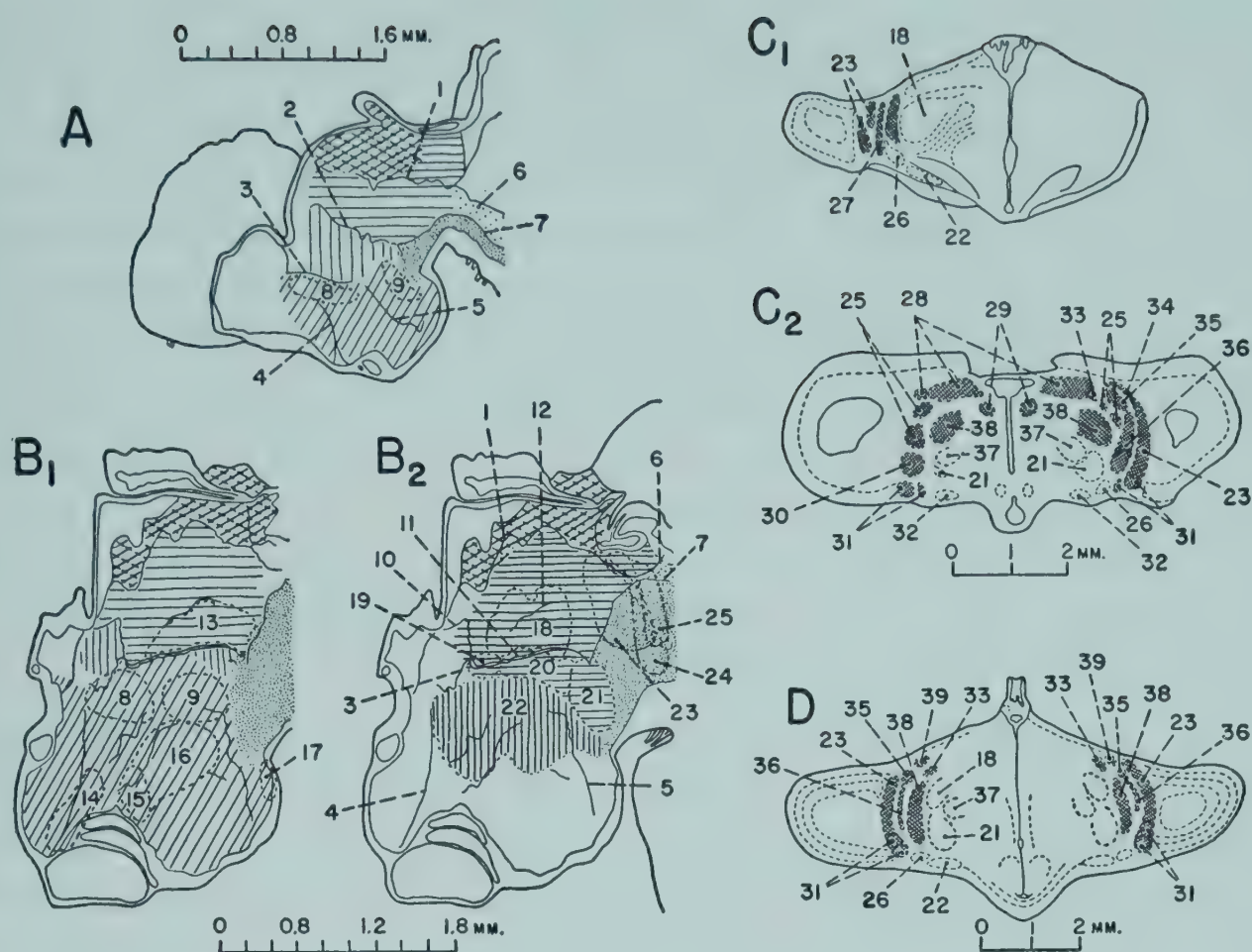
In the dorsal portion of the hypothalamus, directly below the sulcus diencephalicus ventralis, there is found rostrally the massa cellularis reuniens, pars inferior. This includes the matrix of the nucleus entopeduncularis interstitialis. Caudally, between the sulcus lateralis infundibuli and the rostral end of the mesencephalic tegmentum, the primordium of the posterior entopeduncular nucleus has developed (Fig. 108-A).

During the latter half of the fifth day and the entire sixth day, the thickness of the diencephalic wall increases considerably, especially in the region of the dorsal thalamus. The optic fibers and the fibers of the striae medullares are growing dorsocaudally on the external surface of the diencephalon. The optic chiasma develops and the primitive supraoptic decussation starts to differentiate into its dorsal and ventral components.

*At 7 days.* By the seventh day, the differentiation of layers is more distinct in the epithalamus (*Kuhlenbeck, 1937*). The dorsal thalamus is much increased in thickness and bulges into the ventricle; in its more caudal regions, vaguely outlined cell condensations have appeared. These are the first indications of the development of definitive nuclei—the nucleus rotundus laterally, the nucleus ovoidalis medially, and, ventral to the latter, the nucleus subrotundus.

Within the dorsalward extension of the dorsal thalamus, there have suddenly appeared cell condensation fields which are primordia of pretectal nuclei (*Kuhlenbeck, 1939*). These are: (1) the nucleus praetectalis principalis which, in its ventrolateral extension, contains the still undifferenti-





**Fig. 108.** The development of nuclei in the diencephalic and pretectal regions of the chick embryo's brain. (Redrawn with modifications after Kuhlenbeck, 1937, 1939.)

A, B<sub>1</sub>, and B<sub>2</sub>, projections of the nuclei on the ventricular surface, A at 5 days and B<sub>1</sub> and B<sub>2</sub> at 8 days. Medial cell masses are shown in B<sub>1</sub> and lateral cell masses in B<sub>2</sub>. The dorsal thalamus is indicated by horizontal lines, the ventral thalamus by vertical lines, the hypothalamus by diagonal lines, the epithalamus by crossbarring, the mesencephalic tegmentum by close stippling, the torus semicircularis by open stippling. C<sub>1</sub>, C<sub>2</sub>, and D, cross sections showing some of the tectal, pretectal, and mesencephalic tegmental nuclei of the (C<sub>1</sub> and C<sub>2</sub>) 12-day embryo and (D) the newly hatched chick. Section C<sub>1</sub> is anterior to section C<sub>2</sub>. All in scale.

1, sulcus diencephalicus dorsalis; 2, sulcus diencephalicus medius; 3, sulcus diencephalicus ventralis; 4, sulcus intraencephalicus anterior; 5, sulcus lateralis infundibuli; 6, sulcus lateralis mesencephali; 7, sulcus limitans; 8, nucleus entopeduncularis interstitialis; 9, nucleus entopeduncularis posterior; 10, sulcus diencephalicus medius, pars anterior; 11, sulcus parancephalicus; 12, sulcus accessorius thalami dorsalis; 13, nucleus ovoidalis; 14, nucleus praeopticus medialis; 15, nucleus lateralis hypothalami anterior; 16, nucleus lateralis hypothalami; 17, nucleus mammillaris; 18, nucleus rotundus; 19, nucleus lateralis anterior; 20, nucleus subrotundus; 21, nucleus posteroventralis; 22, corpus geniculatum laterale ventrale; 23, nucleus (or nuclei) lentiformis mesencephali; 24, main pretectal group of nuclei; 25, spiriform nuclei; 26, corpus geniculatum laterale dorsale; 27, nucleus laminaris praecommissuralis; 28, nucleus diffusus parvocellularis commissurae posterioris; 29, nucleus interstitialis tegmentalis commissurae posterioris; 30, nucleus subpraetectalis; 31, nucleus parageniculatus tecti optici; 32, nucleus opticus tegmenti; 33, nucleus praetectalis medialis; 34, nucleus praetectalis lateralis; 35, nucleus praetectalis principalis; 36, nucleus interstitialis tractus praetecto-subpraetectalis; 37, nucleus posterointermedius; 38, nucleus principalis praecommissuralis; 39, nucleus areae praetectalis.



ated matrix for (2) the nucleus subpraetectalis and (3) the nucleus interstitialis tractus praetecto-subpraetectalis; (4) the nucleus praetectalis medialis, ventromedial to the nucleus praetectalis principalis; (5) the nucleus areae praetectalis, dorsomedial to the nucleus praetectalis principalis; (6) the nucleus principalis praecommissuralis, ventral to the preceding nuclei; (7) the dorsomedial and (8) the ventrolateral spiriform nuclei, both as yet undifferentiated from a common matrix medial to the main pretectal group; and (9) the nucleus diffusus parvocellularis commissurae posterioris, dorsal to the spiriform nuclei. The last three are intrinsic nuclei of the posterior commissure (*Kuhlenbeck, 1939*).

In the ventral thalamus, the primordia of the lateral geniculate body (pars ventralis), the nucleus ventrolateralis, and the nucleus of the ventral supraoptic decussation have appeared.

In the hypothalamus, between and below the interstitial and posterior entopeduncular nuclei, there is a cell condensation representing the nucleus lateralis hypothalami. The first differentiation of the mammillary nucleus is visible in the wall of the mammillary recess, dorsal to the sulcus lateralis infundibuli. Ventral to this sulcus, the nucleus inferior hypothalami is present, but poorly defined.

*At 8 days.* By the eighth day, the increased thickness of the diencephalic walls indicates a progressive differentiation of cell masses. The longitudinal zones are seen less clearly than on the seventh day, and the diencephalon in cross section is broader and more characteristically avian.

A dense area has appeared at caudal levels of the epithalamus. This represents the lateral habenular nucleus.

In the lateral portions of the dorsal thalamus the following nuclei can now be identified: anteroventrally, the nucleus lateralis anterior; ventrocaudally, the lateral geniculate body (pars dorsalis); dorsocaudally, the nucleus dorsolateralis superficialis and the nucleus tractus septomesencephali. Medially the common matrix of the nucleus dorsolateralis and the nucleus dorsomedialis has appeared, and the newly differentiated nucleus posteroventralis is found merging with the nucleus subrotundus, now in a lateral position. The pretectal cell masses have grown basally and laterally. Fibers connect the nuclei posteroventralis, rotundus, and principalis praecommissuralis with the mesencephalon.

The cell masses of the ventral thalamus have shifted ventrolaterally and no longer reach the ependyma except in two small areas, one anteriorly and one posteriorly. Two new medial cell condensations have appeared; these are the primordial nucleus reticularis (dorsalis and ventralis) and, caudally, the nucleus intercalatus.

In the hypothalamus, a condensation representing the nucleus preopticus medialis has formed in the preoptic region. The anterior end of the nucleus lateralis hypothalami is sending fibers into the dorsal supraoptic decussa-



tion, which also receives fibers from the nuclei reticulares of the ventral thalamus, from the nucleus entopeduncularis interstitialis of the hypothalamus, and from the telencephalon. The mammillary nucleus has become bilateral.

The principal diencephalic and pretectal cell masses are shown projected on the ventricular surface in Fig. 108- $B_1$  and  $B_2$ .

*At 9 days.* The fibers of the habenulo-interpeduncular tract may be seen at caudal levels of the epithalamus of the 9-day chick embryo. The lateral and medial habenular nuclei are present.

In the dorsal thalamus, the nucleus dorsomedialis and the nucleus dorsolateralis have separated from their common matrix. The nucleus dorsomedialis is differentiating further into the nucleus subhabenularis ventral to the medial habenular nucleus and, more ventrally, into the nucleus paraventricularis dorsalis adjacent to the ependymal lining. In the region of the nucleus ovoidalis, still more ventrally, the nucleus paraventricularis intermedius has appeared in a similar paraventricular position. Caudally, the nucleus postero-intermedialis is visible between the nucleus posteroventralis and the nucleus principalis praecommissuralis.

The lateral geniculate body, pars dorsalis, in its anterior portion (subpars intercalaris), consists of a lateral cell plate and a medial cellular mass connected with the nucleus subrotundus by a band of cells which are possibly migrating laterally into the geniculate body (*Kuhlenbeck, 1937*). More posteriorly in the lateral geniculate body, pars dorsalis, there is an aggregation of unmyelinated fibers (neuropile) demarcated medially by the dorsal continuation of the medial portion of the subpars intercalaris. This more caudal part of the lateral geniculate body, pars dorsalis, is the subpars principalis. At its caudal limit it is continuous dorsally with the nucleus praecommissuralis principalis.

The lateroventral migration of the cell masses of the ventral thalamus has continued so that only a narrow cell band remains connected with the ependyma anteriorly at the level of the fast disappearing sulcus parencephalicus. This cell band is probably a transitory stream of cells migrating into the reticular and ventrolateral nuclei (*Kuhlenbeck, 1937*). In the nucleus of the ventral supraoptic decussation a caudal part has differentiated in close relation to a bundle extending to the mesencephalon and containing fibers of the ventral supraoptic decussation, fibers to the nucleus posteroventralis, and fibers traceable to the nucleus subpraetectalis.

In the hypothalamus the following new nuclear masses have appeared: the supraoptic nucleus; the nucleus praeopticus dorsolateralis; the nucleus paraventricularis magnocellularis, extending from caudal parts of the pre-optic region to caudal levels of the hypothalamus; and, farther caudally, the nucleus lateralis hypothalami, between the basal end of the optic tract and the surface of the hypothalamus.



*At 12 days.* The adult nuclear pattern is essentially completed by the twelfth day. At this time, the nucleus praetectalis lateralis and the nucleus laminaris praecommissuralis have appeared in the pretectal region.

A few embryonic features remain. For example, the cells are smaller than in the adult, the fiber systems are not fully developed, a few migrating cell streams are still present, and the subpars intercalaris of the lateral geniculate body, pars dorsalis, exceeds its definitive size. From this time on, however, development consists mainly of growth accompanied by small structural changes.

Cross sections of the pretectal region of the 12-day chick embryo and of the newly hatched chick are presented as Fig. 108-C<sub>1</sub>, C<sub>2</sub>, and D.

### *The Mesencephalon*

The mesencephalon in the bird is characterized by the very large optic tecta. Only in the teleost fish does the development of the tectal regions equal that found in birds. The afferent connections of the tectum are largely with the retina and the efferent connections are with the striatum, so that a great optico-sensory system is formed. The interior of the mesencephalon is concerned with oculomotor functions and with cerebellar and rubrospinal connections for locomotion and flight.

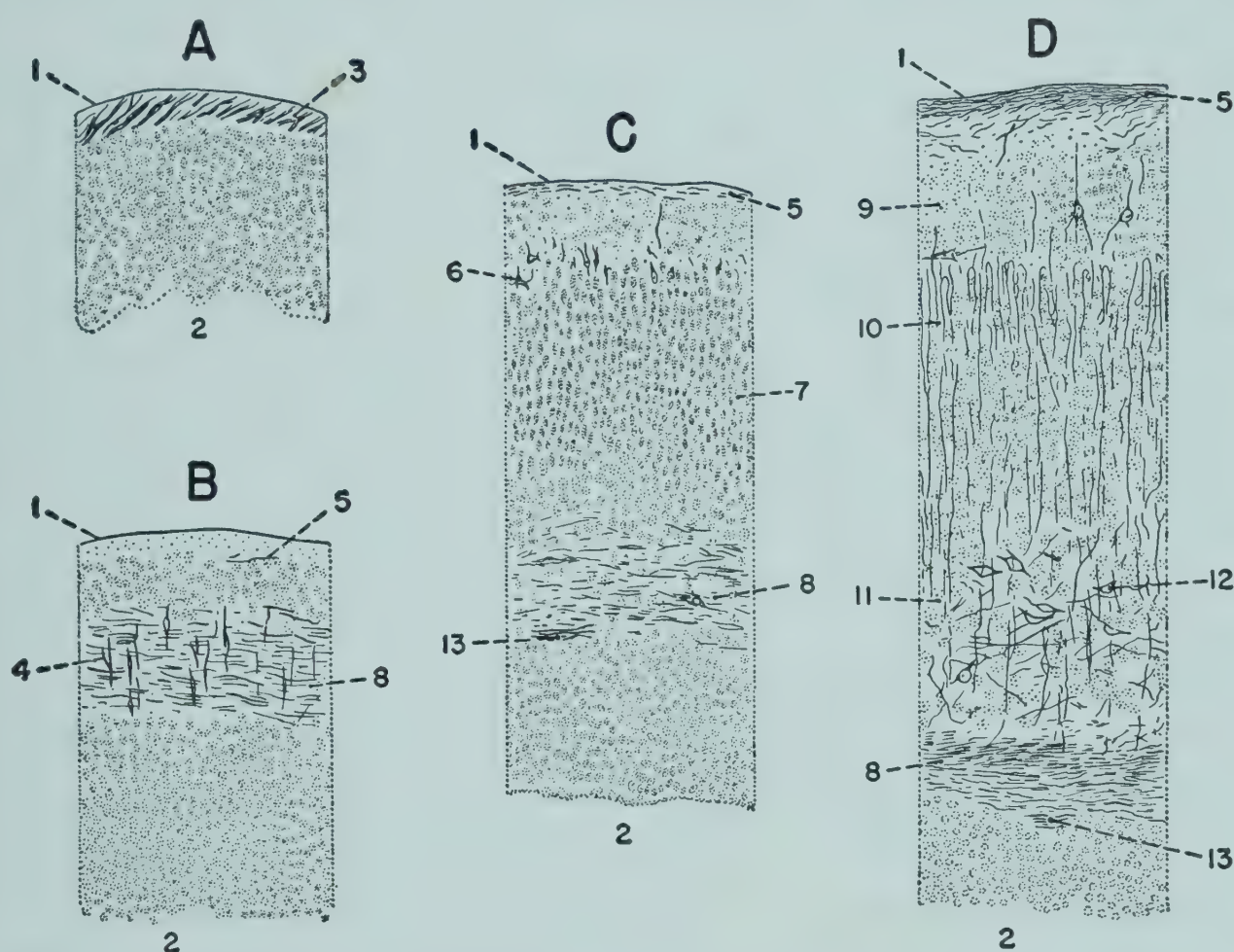
*The optic lobes.* Because the visual apparatus of birds is very well developed, it is not surprising to find that the characteristically laminated structure of the optic lobes is more complex than in reptiles or mammals. Fifteen layers were distinguished by Ramon (1891) in the superior colliculus, or optic tectum, of birds. For convenience, Gehuchten (1892) grouped these layers into three larger zones, as follows: an external zone, containing the first seven layers of Cajal and formed chiefly of the fiber endings of the optic nerve, an intermediate zone of gray substance, consisting of nerve cells and embodying Cajal's layers 8 to 12, inclusive, and an internal zone of white substance, the strata of which form the last three of Cajal's layers. These three strata consist of large ganglionic cells, deep nerve fibers, and central gray substance, respectively. Later, Huber and Crosby (1929) regrouped the tectal layers into six strata, one of which contains six substrata. In the chick embryo, the stratification of the optic lobes is virtually completed by the tenth or eleventh day of incubation (Ramon, 1891; Tello, 1923).

The evolution of the strata of the chick embryo's optic lobes has been described by Tello (1923). The differentiation and migration of neuroblasts in the mesencephalon, already initiated at 52 hours of incubation, has progressed so far by 72 hours that the surface of the mesencephalon is covered with fibers. During the fourth and fifth days, the lamination of the optic lobes begins to appear. The axons of the neuroblasts, directed parallel to the surface of the optic lobe, form a fibrous layer outside of the mantle



layer (Fig. 109-A). This fibrous layer is destined to become the deep fibrous layer (stratum album centrale, stratum medullare profundum, the fourteenth layer of Cajal).

Toward the end of the fifth day, the first fibers from the retina reach the optic lobes. Coincidentally, the cells in the outer portion of the mantle layer, directly beneath the fibrous layer, differentiate as neuroblasts, and the thickness of the fibrous layer is thereby augmented. This process begins anteriorly and basally and spreads upward and posteriorly during the sixth day.



**Fig. 109.** Four stages in the development of the inner structure of the optic lobe of the chick embryo's brain. (Redrawn with modifications after Tello, 1923.)

A, at 5 days; B, at 6 days; C, at 7 days; D, at 11 days of incubation. All  $\times 125$ .

1, surface of optic lobe; 2, ventricular cavity; 3, fibrous stratum; 4, migrating neuroblasts; 5, retinal fibers; 6, previously migrated neuroblasts; 7, undifferentiated cells, previously migrated; 8, deep fibrous layer; 9, sixth layer of Cajal; 10, eleventh layer of Cajal; 11, thirteenth layer of Cajal; 12, large ganglion cells; 13, bundles of large fibers on the internal surface of the deep fibrous layer.

A second process is initiated at the beginning of the sixth day. This process is the migration of undifferentiated and partially differentiated cells of the mantle layer toward the periphery and through the fibrous layer, which therefore seems to move toward the interior (Fig. 109-B). In this way, a stratum of cells is formed peripheral to the fibrous layer. This peripheral cellular zone represents the primordial intermediate zone of gray substance. External to it there is only a narrow zone with rare cells, although



a few retinal fibers paralleling the surface may be found in the vicinity of the diencephalon.

On the seventh day, the thickness of the intermediate zone has increased and begins to exceed that of the zone lying next to the ventricular surface (Fig. 109-C). This increase is due to the accelerated migration of undifferentiated cells into the intermediate zone, which now consists of three layers—a narrow outer layer composed of differentiating cells, a wide median layer, and a narrow inner layer. In addition, the external zone of retinal fibers has started to form, and its inner region shows some slight differentiation into Cajal's sixth layer. This layer can be seen clearly on the ninth day.

By the eleventh day, continued differentiation has added to the complexity of both the external and the deep zone, in the latter of which the layer of large ganglionic cells has appeared (Fig. 109-D). A number of the finer strata are differentiated subsequently.

*Pretectal nuclei of mesencephalic origin.* The pretecal region contains nuclei which are of mesencephalic rather than diencephalic origin (Kuhlenbeck, 1939). These may be divided into a tectal and a mesencephalic tegmental group. Both have appeared in the chick embryo of 7 days' incubation. The matrix of the nucleus lentiformis mesencephali is found in the zone of transition between the optic tectum and the dorsal thalamus. At the ventrolateral and caudal end of this cell condensation is the precursor of the nucleus parageniculatus tecti. These nuclei belong to the tectal group. The dorsolateral end of the tegmental cell cord contains the primordium of the interstitial tegmental nucleus of the posterior commissure. In the 12-day embryo, the nucleus lentiformis mesencephali has differentiated into a pars magnocellularis and a pars parvocellularis.

The appearance of the nuclei in the pretecal region of the 12-day embryo's brain is shown diagrammatically in Fig. 108-C<sub>1</sub> and C<sub>2</sub>, and Fig. 108-D depicts them in the newly hatched chick.

### The Spinal Cord

The spinal cord of birds extends throughout the entire vertebral canal, and there is neither a cauda equina nor a pronounced filum terminale. In external appearance, the cord resembles that of other vertebrates. It is an attenuated organ, somewhat thicker at thoracic and lumbosacral levels than elsewhere, but otherwise tapering slightly from anterior to posterior. It also exhibits one or two characteristically avian features. In most birds, its cervical portion is relatively long; and there is a distinctive structure incorporated within the lumbosacral enlargement. This structure is the glycogen body, which differentiates within the roof plate during the embryonic period and spreads the two sides of the cord apart dorsally to form the sinus rhomboidalis.



In cross section, the cord is thick-walled and composed of the irregularly H-shaped gray substance (nerve cell bodies and unmyelinated fibers) internal to the white substance (myelinated fibers). The two lateral halves of the cord are separated dorsally by the dorsal septum and ventrally by the median ventral fissure and are continuous with each other only in a small region surrounding the minute central canal.

The gray matter, on each side, is composed of the dorsal and the ventral horn or column. The dorsal horn is primarily sensory in nature and contains the nuclei of termination of the dorsal root fibers in the spinal nerves, which originate in the spinal ganglia outside the cord. The ventral horn is motor in nature, and in it are found the cells of origin of the ventral roots of the spinal nerves. In the mediodorsal portion of the gray substance at thoracic, upper lumbar, and sacral levels arise visceral motor fibers; these leave the cord by most of the ventral nerve roots and proceed via the rami communicantes to the paravertebral chains of autonomic ganglia. Between the dorsal and ventral horns are neurones which send their fibers into the white matter to ascend or descend for short distances, thus to correlate spinal reflexes.

The white matter of the cord consists of three large divisions on either side. Between the dorsal septum and the dorsal horn of the gray substance is the dorsal white column; the lateral white column lies between the dorsal and ventral horns of the gray substance; and the ventral white column between the ventral horn and the median ventral fissure. These columns of white matter contain ascending and descending fiber tracts, the shorter tracts in general lying closer to the gray substance.

Collateral fibers place the gray and white substance in communication. The two longitudinal halves of the cord are connected by gray and white commissures crossing the midline.

### *Gross Morphological Development of the Spinal Cord*

The rudiment of the spinal cord can be considered as completely formed when the caudal end of the primitive neural tube has been laid down by the tail bud, although, as noted previously, the last of the neural material proliferated from this source is resorbed and does not take part in the formation of the spinal cord. In the meantime, the structural differentiation of the spinal cord has begun at the anterior end and is proceeding caudalward.

Since the first five somites disappear in the chick (*Williams, 1910*), and the first cervical ganglion forms between the fifth and sixth somites (*Yntema and Hammond, 1945*), the boundary between the medulla oblongata and the spinal cord probably falls at the level of the fifth somite. *Holmdahl (1928)*, however, concluded that the transition from brain to spinal cord is made in passing from the third somite to the fourth.

Aside from size increase, the chief changes characterizing the gross development of the spinal cord in birds are the thickening of the lateral walls



of the neural tube, especially in its ventral half, and the near-obliteration of the central canal. As in other vertebrates, the cord becomes especially thick in the thoracic and lumbosacral regions.

According to the account given by Kupffer (1906), the neural tube begins to grow dorsoventrally as soon as it is separated from the ectoderm, so that its height exceeds its width and its central canal is transformed into a slit. The dorsal and ventral walls then become much thinner than the lateral walls and thus form the early roof and floor plates. The greatest dorsoventral growth occurs before the end of the chick's fourth day of incubation. There is little or no subsequent increase in the dorsoventral diameter of the gray matter, although the addition of white matter continues to augment this diameter of the cord for several days. Growth of the spinal cord after the fourth day, however, is mainly lateral and longitudinal (*Hamburger, 1948*).

TABLE 5  
Size of the Developing Lumbosacral Spinal Cord and  
of the Central Canal in the Chick Embryo \*

Age of Embryo	Spinal Cord			Central Canal	
	Height	Width	H/W	Height	Width
(days)	(mm.)	(mm.)		(mm.)	(mm.)
6	0.385	0.290	1.30	0.300	0.045
7	0.530	0.475	1.10	0.365	0.035
8	0.700	0.800	0.88	0.400	0.040
9	0.800	1.100	0.70	0.400	
10	0.920	1.160	0.80	0.260	
11	0.970	1.200	0.80	0.180	0.080
12	1.100	1.400	0.78		
13	1.230	1.800	0.68		
14	1.390	1.860	0.74		
15	1.620	2.100	0.77		
16	1.890	2.380	0.79		
17	1.900	2.400	0.79	0.075	0.026
18	1.960	2.610	0.75	0.030	0.023
19	2.030	3.150	0.64	0.014	0.011

\* After Imhof (1905).

As the lumen of the cord grows smaller, it loses its slitlike form and becomes almost circular. The reduction in the size of the central canal occurs as the dorsal median septum is formed by a lengthening of the fibers of the roof plate, a process that is perhaps accompanied by the fusion of the lateral walls of the canal or by a contraction of its inner limiting membrane.



The permanent spinal canal represents only the ventralmost portion of the original slitlike lumen of the cord. At brachial levels, the size of the central canal diminishes gradually from the fifth to the seventh day and rapidly from the seventh to the ninth day (Hamburger, 1948). Clarke (1862) observed that there is no dorsal median septum and only a trace of the ventral median fissure in the spinal cord of the 5-day chick embryo, but that both septum and fissure are well developed by the ninth day of incubation.

Imhof (1905) described the gross appearance of the spinal cord of the chick embryo from the sixth to the nineteenth day of incubation. Table 5 gives Imhof's figures for the height and width of the spinal cord and the central canal. Some indication of the progressive development of external features of the spinal cord may be seen in Fig. 110, which shows the ap-

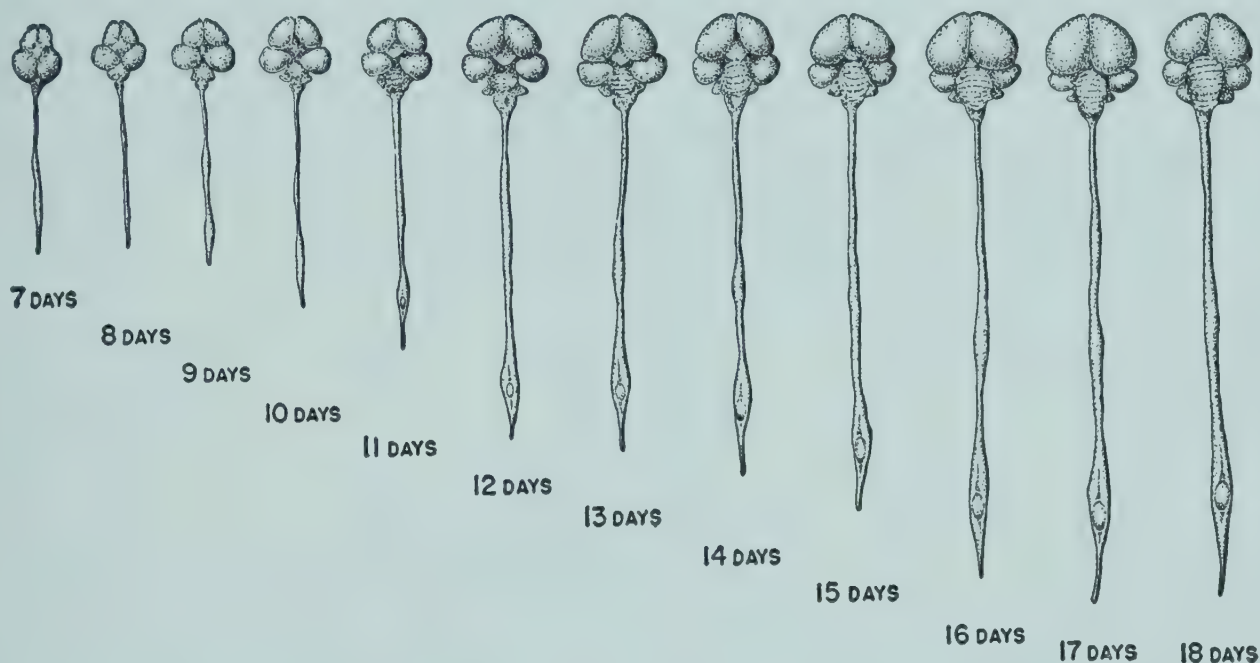


Fig. 110. Dorsal views of the central nervous system of the chick embryo from the seventh to the eighteenth day of incubation, inclusive, showing the growth of the cord and the development of the brachial and lumbosacral enlargements and the glycogen body. All  $\times 0.5$ . (Redrawn after Watterson, 1949.)

pearance of the chick embryo's central nervous system from the seventh to the eighteenth day of incubation.

**The glycogen body and the sinus rhomboidalis.** The literature concerning the development of the sinus rhomboidalis in the lumbosacral region of the spinal cord and of the jelly-like material that fills this space has been reviewed by Imhof (1905). According to Imhof's account, Steno (1667) first described the lumbosacral enlargement and a "cavitas rhomboidalis" in the spinal cord of the bird. Imhof stated that Jacobaeus (1675) introduced the term "sinus rhomboidalis sacralis."

The sinus rhomboidalis is normally filled with a very fragile, ovoid cellular mass (the "glycogen body"), which is actually an integral part of the spinal cord although it is so closely associated with the pia mater that it is usually removed with the leptomeninges. Thus the sinus is actually an



artifact. The cellular mass has been referred to as the "gallertiges wasserhelles Kügelchen" (Emmert, 1811), "eiweissähnliche Flüssigkeit" (Nicolai, 1812), "lymphatischen Flüssigkeit" (Leydig, 1854), "Gallertgewebe" (Stieda, 1869), "sostanza gelatinosa rhomboidali" (Lachi, 1889), "Gliawulst" or "dorsale Gliawucherung" (Kölliker, 1902), "Lumbalwulst" or "lumbalen Gliawulst" (Imhof, 1905), "glycogenic body" (Terni, 1924a), and "glycogen body" (Watterson, 1949; Doyle and Watterson, 1949; Watterson and Spiroff, 1949).

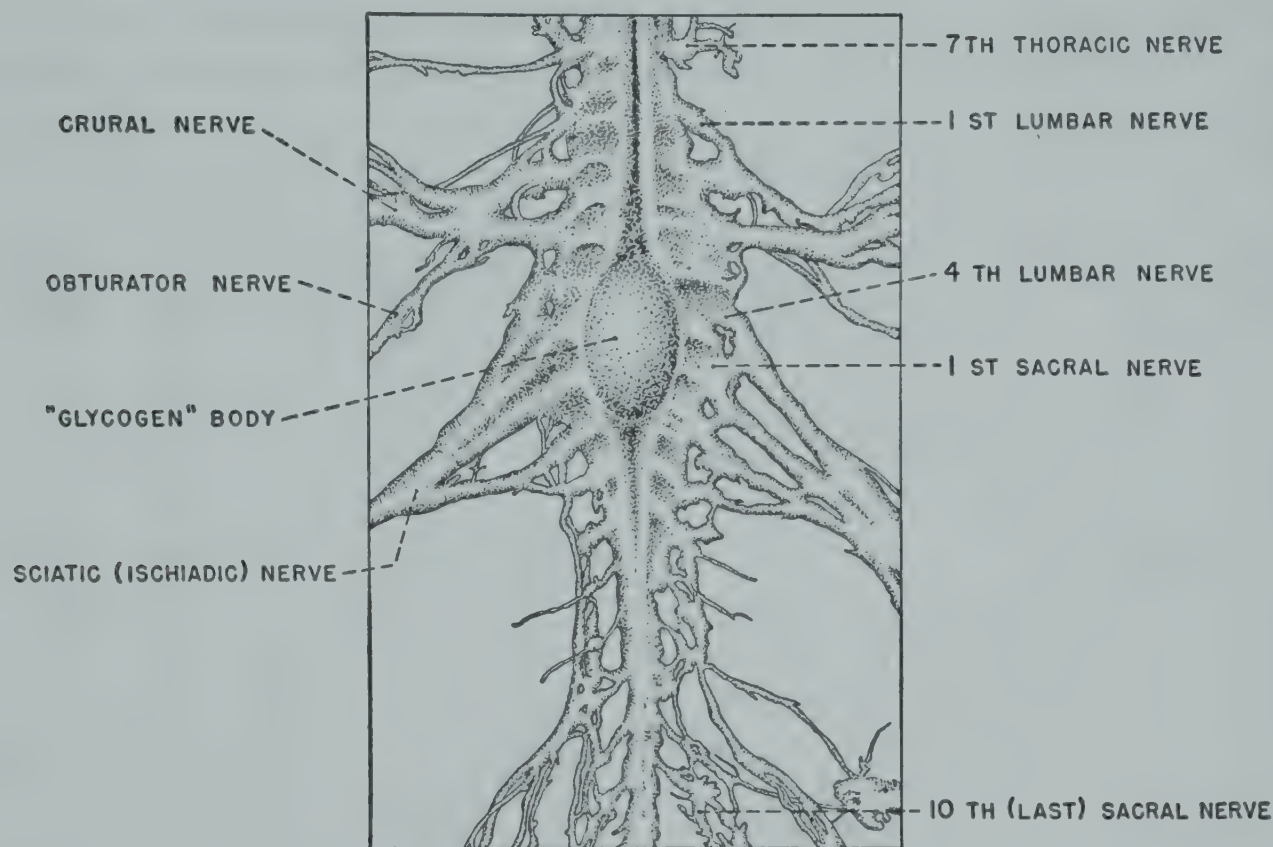


Fig. 111. A dissection of the spinal cord and the lumbosacral plexus of a newly hatched chick;  $\times 2$ . (Redrawn with modifications after Watterson, 1949.)

The gross appearance of the structure in the ostrich (*Struthio camelus*) has been described by Streeter (1904); in a flamingo bird (*Phoenicopterus ruber*) by Imhof (1905); in the pigeon (*Columba livia*) by Huber (1936); and in the chicken (*Gallus gallus*) by Watterson (1949). Figure 110 shows the progressive development of the lumbosacral enlargement and the glycogen body in the spinal cord of the chicken, and their appearance soon after hatching may be seen in Fig. 111.

The differentiation of cells in the lumbosacral region of the spinal cord of the chick embryo shows no unusual features until the eighth day (Watterson, 1949) or ninth day (Imhof, 1905) of incubation. According to Watterson (1952) paired primordia can be demonstrated at 7.5 to 8.17 days' incubation lying to each side of the ependymal septum of the roof plate of the neural tube. Fusion of these paired primordia occurs in dorsoventral sequence between 8.17 and 9 days' incubation, fusion becoming possible as the ependymal septum degenerates or thins out (Watterson, 1954).



From the eighth to the fifteenth day there is an intensive differentiation of special cells, which are able to store glycogen intracellularly, in the lateral margins of the roof plate of the last lumbar and first three sacral segments (Watterson, 1949). This phase of proliferation reaches its peak at 10.75 days of incubation (Watterson, 1949), and thereafter the increase in the size of the cells is principally responsible for the enlargement and protrusion of the glycogen body. Imhof (1905) stated that the most intensive period of growth was during the thirteenth day of incubation. The elevation of

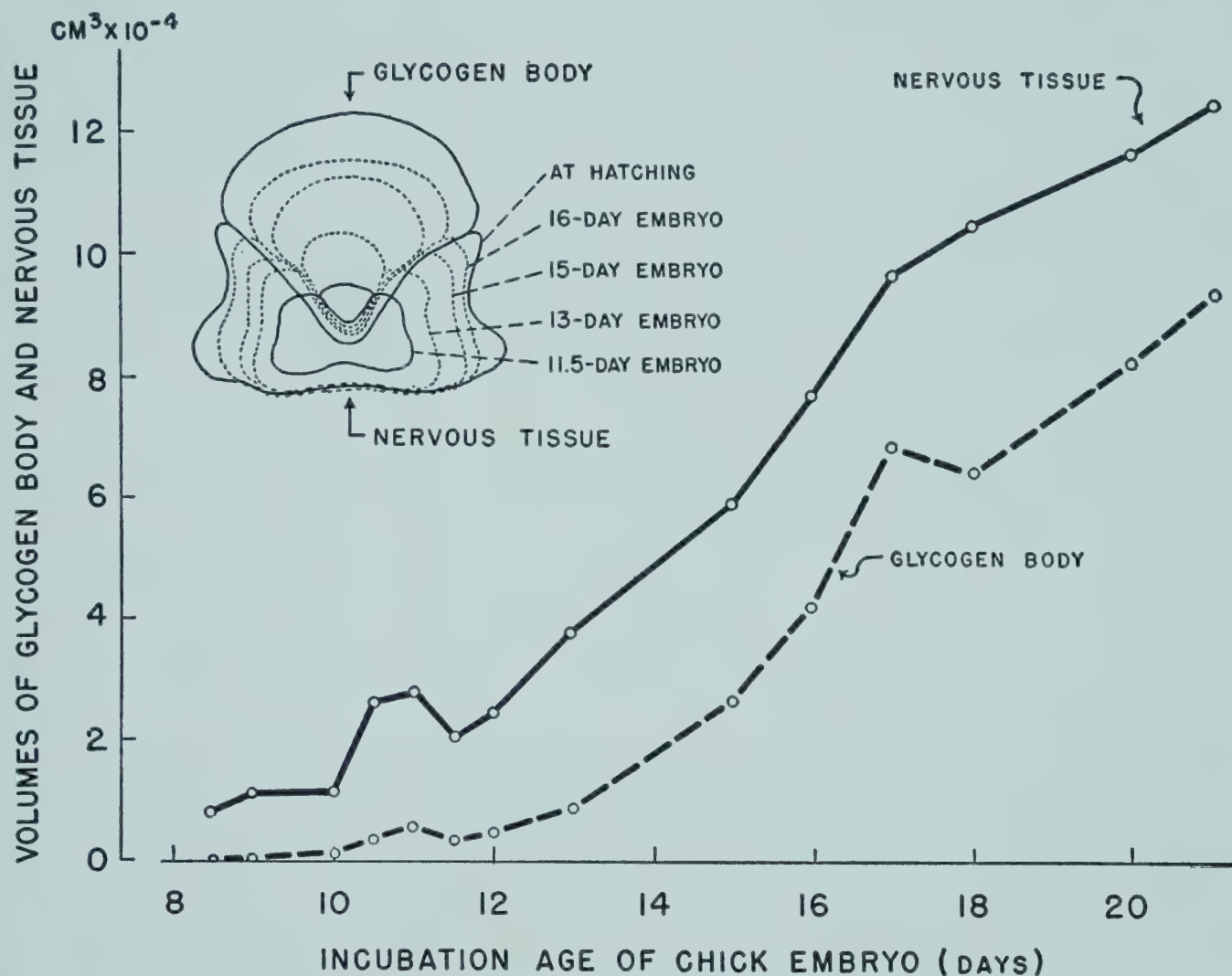


Fig. 112. The growth and development of the glycogen body in the lumbosacral region of the chick embryo's spinal cord. (Redrawn with modifications after Watterson, 1949.)

The increase in the volume of the glycogen body (broken line) is compared with the increase in the volume of adjacent nervous tissue (solid line) from the eighth day of incubation to hatching.

*Insert:* superimposed outline drawings of cross sections of the lumbosacral spinal cord at the level of the glycogen body, made at successive stages of incubation ( $\times 8$ ).

the glycogen body carries the pia mater dorsally, and it is associated also with the spreading of the dorsal white columns laterally from the last thoracic segment to the seventh sacral segment (Watterson, 1949). By the fourteenth or fifteenth day of incubation the glycogen body completely surrounds the central canal, and the two halves of the spinal cord are connected by the ventral commissure alone (Watterson, 1949). The progressive enlargement of the glycogen body is shown in cross section in Fig. 112,



which compares the growth of the glycogen body with that of the adjacent nervous tissue.

The glycogen body becomes visible externally between the tenth and eleventh days of incubation and increases gradually in anteroposterior extent until the twentieth or twenty-first day (Watterson, 1949). The rapid increase in the size of the structure and of its cells coincides with the increase in intracellular glycogen. Segments 26 to 29 of the spinal cord of the chick embryo contain approximately 0.012 mg. of glycogen on the tenth day of incubation; 0.373 mg. on the fourteenth day; and 3.0 mg. at the time of hatching (Doyle and Watterson, 1949). This represents 5 to 10 per cent of the total body glycogen from the fifteenth day to hatching; and, unlike the liver glycogen, this store is not depleted at hatching or by starvation for 24 hours after hatching (Doyle and Watterson, 1949).

Origin of glycogen-storing cells from dorsomedian margins of the inner mantle and from the dorsalmost portions of the neural epithelium to each side of the ependymal cells of the roof plate is postulated by Watterson (1952).

The function of the glycogen body and its large store of glycogen is not clear. Reduction of the periphery exerts little effect upon the development of this mass. The factors which influence its development appear to be intraneural or, if they lie outside the neural tube, must be in either the somites, the notochord, or the neural crest immediately adjacent to it (Watterson and Spiroff, 1949).

**Hofmann's nuclei.** Gaskell (1889) and Lachi (1889) described a segmentally arranged series of projecting nuclei in the lumbosacral region of the dove. Kölliker (1902) published an excellent account of these nuclei in the dove and chicken and named them Hofmann's nuclei. Five or six pairs are found in the lumbosacral region of the chick's spinal cord. Streeter (1904) observed 6 pairs from the eighth lumbar to the sixth sacral segment of the spinal cord of the ostrich (*Struthio camelus*), and termed them major marginal nuclei. Huber (1936) observed them in the pigeon (*Columba livia*) and considered them a part of the marginal paragriseal column. Imhof (1905) noted that, in the chick embryo, these nuclei are differentiated from the mantle layer on about the sixth day of incubation, and that they begin to protrude on the surface of the cord at about the fourteenth day.

### *Cytoarchitectural Development of the Spinal Cord in the Chick Embryo*

The derivation of the columns of the spinal cord from various portions of the basal and alar plates of the neural tube has been shown by Wenger (1950). The ventralmost portion of the basal plate gives rise to the region between the medial motor column and the floor plate; the ventrolateral portion of the basal plate becomes the medial motor column; the lateral



basal plate forms the lateral motor column; and the dorsal basal plate differentiates as the intermediate or internuncial region (formed of connecting cells) of the ventral horn. The alar plate provides the dorsal horn and the dorsal portion of the intermediate (internuncial) region of the central gray substance.

The roof and floor plates of the chick's spinal cord have differentiated after about 2.5 days of incubation. At this time, the basal and alar plates consist chiefly of the neural epithelium with its inner germinal zone of dividing cells, but a narrow marginal layer can be distinguished on the lateral circumference of the cord. At the 3-day stage, the mantle layer becomes identifiable between the neural epithelium and the marginal zone. The marginal layer now extends around the ventral and ventrolateral circumference of the cord; it completely surrounds the gray matter after 6 days of incubation. The definitive ependymal layer appears in the region of the basal plate after 5 to 8 days' incubation and in the ventral part of the alar plate at the 8-day stage. It is formed by the migration of oblong nuclei into the fibrous germinal zone from more peripheral regions of the neural epithelium. Between the eighth and ninth days of incubation, the germinal zone adjacent to the roof plate stretches, instead of being invaded by nuclei, and the radial fibers of this zone join those of the roof plate to form the dorsal median septum (*Hamburger, 1948*).

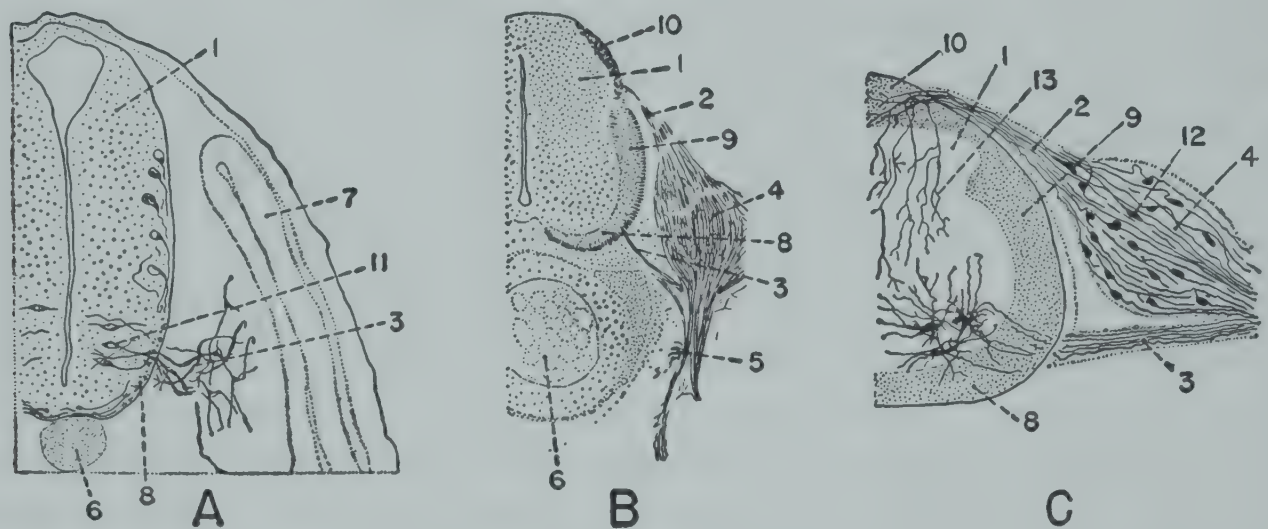
The development of the spinal cord is the result of the proliferation, migration, and differentiation of cells. Proliferation (that is, cell division) is limited to the germinal zone of the neural epithelium (*Ramon, 1890b*), but the dividing cells are probably derived from overlying zones of this layer (*Hamburger, 1948*). Proliferation occurs as a wave of cell division that spreads in a ventrodorsal direction, from basal to alar plate, and from cephalic to caudal levels of the cord. The number of mitoses reaches a peak in the basal plate on the third day of incubation and in the alar plate on approximately the sixth day. The motor portion of the cord thus develops before the sensory portion. The main period of mitotic activity is past after 8.5 days, and cellular proliferation then declines steadily, to terminate by the hatching date (*Hamburger, 1948*).

Proliferative activity in the chick's spinal cord is accompanied and succeeded (1) by the differentiation of some cells of the neural epithelium into primary neuroblasts and their subsequent migration into the mantle layer, and (2) by the migration of undifferentiated cells into the mantle layer, where they later differentiate as secondary neuroblasts. The early primary neuroblasts have both a distal process (axon) and a central process. As the cell migrates peripherally, the central process atrophies or retracts and is replaced by a new outgrowth, so that the cell again becomes bipolar or, eventually, multipolar (*Ramon, 1890b; Windle and Austin, 1936*). Indifferent cells that have migrated into the mantle layer begin their differ-



entiation as secondary neuroblasts after the definitive dendrites have appeared on the primary neuroblasts; that is, at the time that the axons of the latter cells reach the periphery (*Ramon, 1890b; Hamburger and Keefe, 1944; Barron, 1946*) cells are present in the ventral horn of the central gray (*Ramon, 1890b*).

From the ninth to the twelfth or thirteenth days of incubation, there is a great increase in the complexity of the dendritic arborizations of the cells of the central gray of the spinal cord, and the neuropile (a network of very



**Fig. 113.** The development of the dorsal and ventral spinal nerve roots and the dorsal root ganglion in the chick embryo. (Redrawn with modifications A, after Tello, 1923; B, after Windle and Orr, 1934; C, after Ramon, 1890a.)

A, a transverse section of the cervical spinal cord of a 46- to 48-hour chick embryo, showing the emergent ventral root fibers ( $\times 200$ ); B, a transverse section of the cervical spinal cord (seventh segment) of a 6.5- to 7-day chick embryo, showing the dorsal and ventral spinal roots, the dorsal root ganglion, and a distinct marginal layer ( $\times 50$ ); C, a transverse section of the thoracic spinal cord of a 9-day embryo, showing the dorsal and ventral spinal roots and the cellular pattern in the dorsal root ganglion and in the ventral motor column by the Golgi method ( $\times 50$ ).

1, spinal cord; 2, dorsal spinal root; 3, ventral spinal root; 4, dorsal root ganglion; 5, secondary sympathetic ganglion; 6, notochord; 7, myotome; 8, ventrolateral white column; 9, lateral white column; 10, dorsal white column; 11, motor neuroblasts; 12, sensory ganglion neuroblasts; 13, collaterals to the central gray from the sensory fibers in the dorsal white columns.

short, unmyelinated fibers) of this region begins to assume its adult appearance (*Ramon, 1890b; Zurabashvili, 1943a*). The general appearance of the roof and floor plates, and of the basal and alar plates in the lumbar cord of the 6- to 19-day chick embryo, has been fully discussed by Imhof (1905).

**The First 4 Days.** The cytoarchitectural changes that occur in the spinal cord are the result of two processes: (1) the development of neurones, and (2) the development of the fiber systems and the neuropile (*Zurabashvili, 1943b*). The development of neurones precedes, of course, the development of fibers, but soon both processes overlap.

In the upper cervical region of the spinal cord of the 46- to 48-hour chick



embryo (above cervical segment 5), Tello (1923) observed longitudinally coursing fibers in the ventral white columns, a number of commissural fibers, several motor neuroblasts with a bipolar appearance, and a ventral root. The peripheral course of the latter fibers is disoriented (Fig. 113-A). Below the fifth cervical segment the longitudinal tract disappears and ventral commissure fibers are infrequent. These observations agree with those of Windle and Austin (1936), who stated that motor neuroblasts are present as far caudad as the twenty-sixth somite (about the sixth thoracic seg-

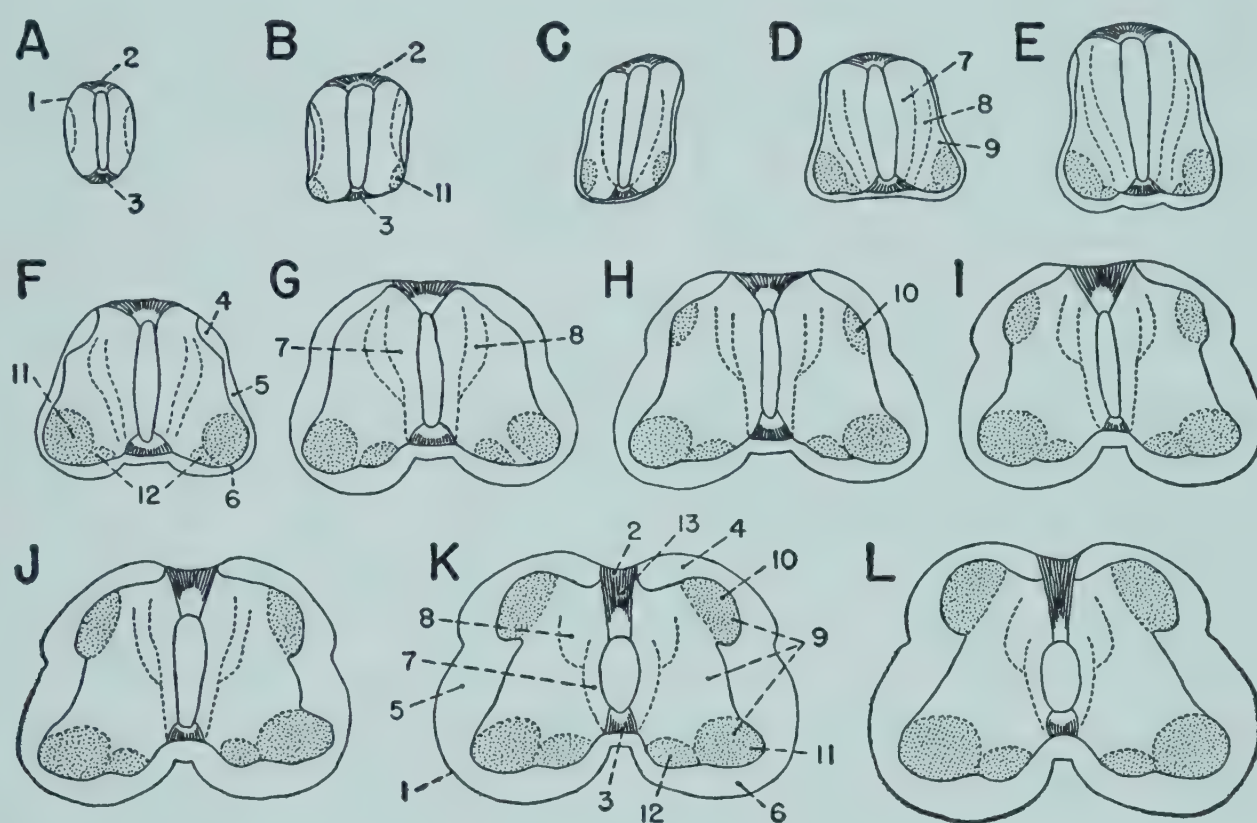


Fig. 114. The progressive development of the various portions of the white and central gray columns of the spinal cord in the chick embryo of 2.5 to 8.5 days' incubation, as shown in diagrammatic representations of cross sections through the cord (fifteenth segment). (Redrawn after Hamburger, 1948.)

A, at 2.5 days; B, at 3 days; C, at 3+ days; D, at 4 days; E, at 4+ days; F, at 5 days; G, at 6 days; H, at 6.5 days; I, at 7 days; J, at 7.5 days; K, at 8 days (lumbosacral region); L, at 8.5 days of incubation. All  $\times 50$ .

1, external limiting membrane; 2, roof plate; 3, floor plate; 4, dorsal white column; 5, lateral white column; 6, ventral white column; 7, ependymal zone; 8, inner mantle zone; 9, outer mantle zone; 10, dorsal gray column (horn); 11, lateral motor column (horn); 12, medial motor column; 13, region of specialized cells of the roof plate which will form the glycogen body.

ment). After 60 hours of incubation, motor neuroblasts have been observed as far caudad as the last (thirty-second) somite, or about at the level of the first sacral segment (Windle and Austin, 1936). Figure 114-A shows an outline drawing of the fifteenth cervical segment of the spinal cord after 2.5 days of incubation. The cord is oval, a sulcus limitans is identifiable, and the mantle layer is as yet unformed. The floor and roof plates are separate from the lateral walls. In the lateral walls a germinal layer, a middle nu-



cleated layer, and an outer marginal velum are identified. In the ventrolateral portion of the basal plate, the cells are irregularly arranged and more crowded. This is the early stage of the lateral motor column (*Hamburger, 1948*).

After 72 hours of incubation the cervical cord contains a large and well-developed lateral motor column (*Ramon, 1890b; Hamburger, 1948*). Golub (*1934*) described a well-developed lateral motor column in the spinal cord of the 78-hour chick embryo, as well as a ventral motor root and a poorly differentiated intermediate region of the central gray. The fibers of the lateral motor column cells exit principally in the ventral root, although a few fibers pass into the dorsal root. Cells of the latter type occur only in the cervical cord (*Tello, 1923*). The first sensory root fibers reach the surface of the cord (*Tello, 1923*), but no dorsal white columns have been formed (*Windle and Austin, 1936*). The thoracic and lumbar cord at this time has the appearance of the cervical cord at 48 hours (*Tello, 1923*). Figures 114-B and C are outline drawings of the fifteenth cervical segment of the cord at this stage, and Fig. 115-A shows the cellular picture. The ependymal and mantle layers are identified and the lateral and ventral white columns have appeared.

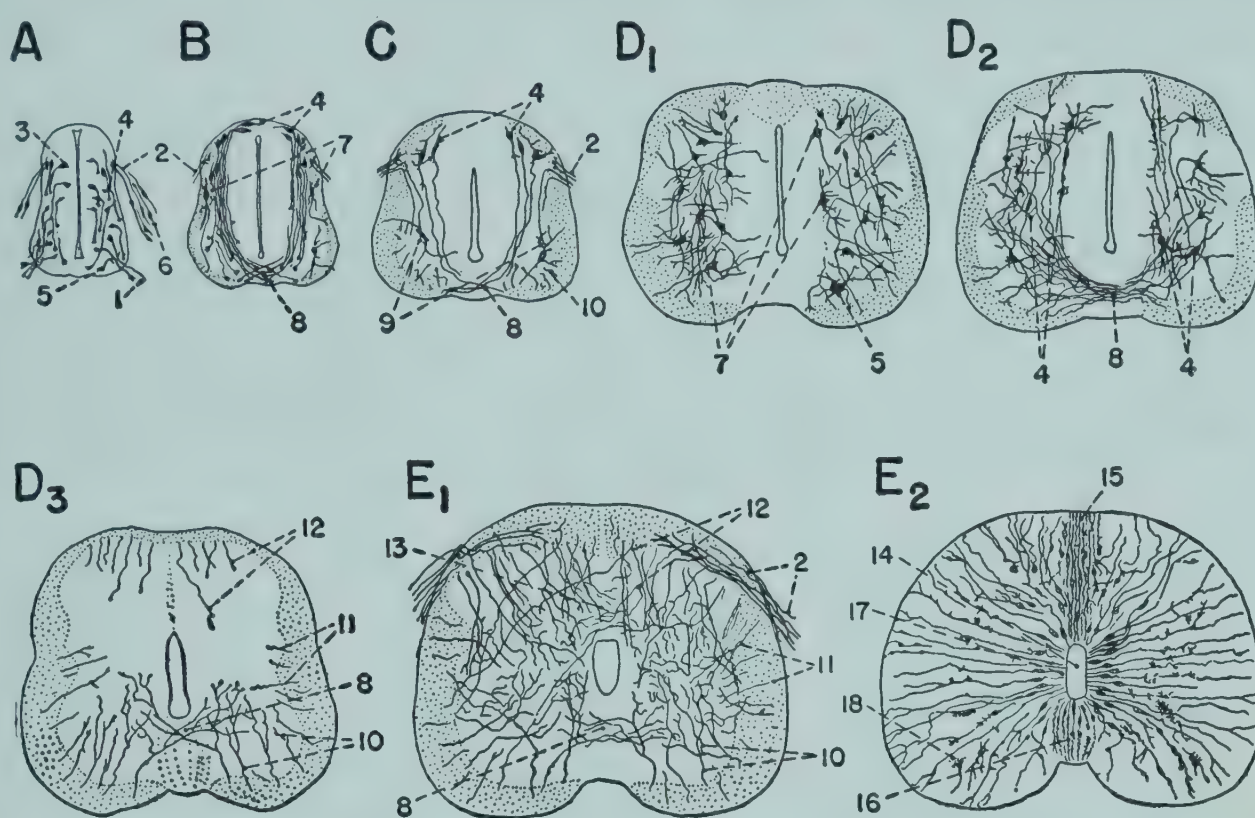
Commissural and associational cells, mostly still unipolar (*Ramon, 1890b*) (cf. Fig. 115-A), are appearing in the alar and dorsal part of the basal plate (*Windle and Austin, 1936; Hamburger, 1948*). The fibers of these cells will form the ipsi- and contralateral lateral and ventral white columns (*Windle and Austin, 1936*). Ventral commissural fibers extend only a short distance rostrally and caudally in the cord (*Ramon, 1890b*), and the size of the ventral white columns varies directly with the size of the commissure (*Windle and Austin, 1936*).

The appearance of the spinal cord of the chick embryo after 4 days' incubation is seen in Fig. 114-D and E and in Fig. 115-B. The lateral motor columns are well developed (*Hamburger, 1948*) and many of the cells have large dendritic arborizations (*Ramon, 1890b*). There are more cells in the intermediate region of the central gray than at 72 hours; many of them are commissural (*Hamburger, 1948*), and some are cells of the ventral and lateral white columns (*Ramon, 1890b*). A small dorsolateral white column has appeared (*Hamburger, 1948*). An abortive visceral motor column with its cells still mingled with the ventrolateral motor column has been identified in the cervical region on the fourth day and is possibly present on the third day. The neurones of this system degenerate by the fifth day (*Levi-Montalcini, 1950*).

**At 4.5 to 5 Days.** At this time the stage of proliferation and migration of cells is rapidly waning in the basal plate and increasing in the alar plate. The basal plate has thus already acquired its basic structural pattern, whereas the alar plate shows no typical features as yet (*Hamburger, 1948*).



Figure 114-F shows the general outlines of the regions of the brachial spinal cord at the 5-day stage. Figure 115-C and Fig. 116-A<sub>1</sub> and A<sub>2</sub> show the cellular and fiber patterns at the same stage. The basal plate contains large multipolar neuroblasts which are somatic motor and (in the thoracolumbar and sacral regions) special visceral motor also (*Windle and Orr, 1934; Levi-Montalcini, 1950*). These cells send their axons into the ventral roots;



**Fig. 115.** The progressive cytoarchitectural development of the chick embryo's spinal cord, as shown in cross sections stained by the Golgi method. (Redrawn with modifications A, B, C, D<sub>3</sub>, after Ramon, 1890b; D<sub>1</sub>, D<sub>2</sub>, E<sub>1</sub>, E<sub>2</sub>, after Ramon, 1890a.)

A, the pattern of neuroblasts at 3 days; B, the pattern of neuroblasts in the thoracic spinal cord at 4 days; C, early commissural neuroblasts and the neuroblasts of origin of efferent dorsal root fibers at 5 days; D<sub>1</sub>, associational neuroblasts at 7 days; D<sub>2</sub>, commissural neuroblasts at 7 days; D<sub>3</sub>, collaterals to the central gray from the white columns at 7 days; E<sub>1</sub>, collaterals to the central gray from the white columns at 9 days; E<sub>2</sub>, neuroglia and ependymal cells at 9 days. All  $\times 60$ .

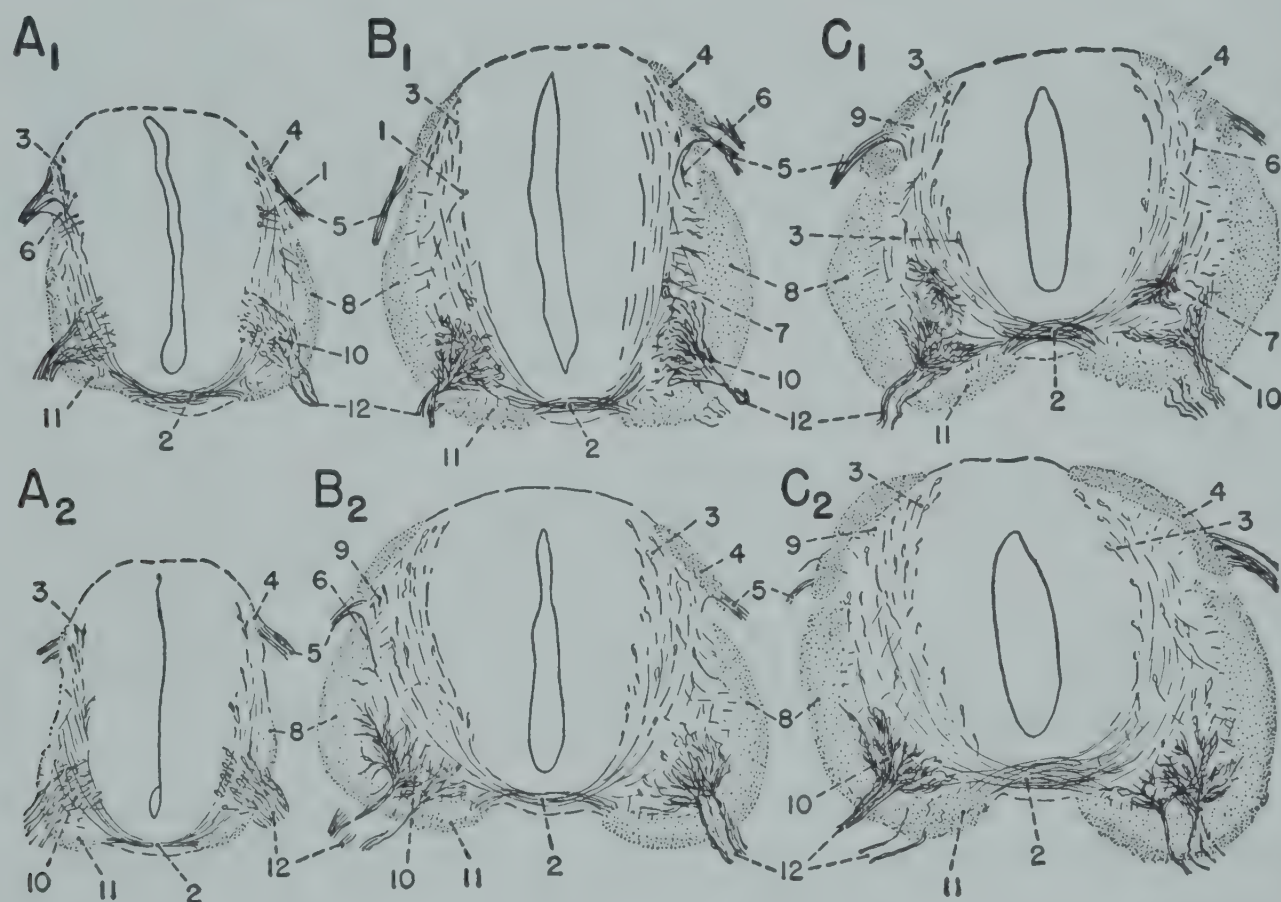
1, ventral root; 2, dorsal root; 3, early neuroblast; 4, commissural neuroblast; 5, motor neuroblast; 6, ganglion neuroblast; 7, associational neuroblast; 8, ventral commissure; 9, ventral horn cell with its axon entering the dorsal root; 10, collaterals to the central gray from the ventral white columns; 11, collaterals to the central gray from the lateral white columns; 12, collaterals to the central gray from the dorsal white columns; 13, dorsal commissure; 14, central canal; 15, dorsomedial fissure at the terminus of the ependymal cells of the roof plate; 16, ependymal cells of the floor plate; 17, ependymal cells of the lateral walls of the neural tube; 18, spongioblasts.

however, a few of them send their fibers dorsally and into the dorsal roots, as is shown in Fig. 115-C (*Ramon, 1890a, 1890b; Lenhossék, 1890; Windle and Orr, 1934*). The dendrites of these cells are very prominent and are spread out toward the ventral commissure (*Lenhossék, 1890*). Windle and Orr (1934) suggested that these cells are a part of the spinal accessory (eleventh cranial nerve) system. Most of these fibers are found in the



upper cervical segments, although some are found as far caudad as the lumbosacral enlargement.

In the alar plate, and to a lesser degree in the basal plate, are seen many unipolar and early bipolar (*Ramon, 1890b*) secondary neuroblasts (*Windle and Orr, 1934*). The axons of these cells pass ventrally, and the majority of them cross in the ventral commissure and enter the ventral white columns of the contralateral side, where they turn rostrad (cf. Fig. 115-C; Fig.



**Fig. 116.** Neurofibrillar structures in the spinal cord of the chick embryo, as seen in cross sections stained by the Pyridine silver method. (Redrawn with modifications after Windle and Orr, 1934.)

A<sub>1</sub> and A<sub>2</sub>, at 5 days of incubation; B<sub>1</sub> and B<sub>2</sub>, at slightly more than 6 days; C<sub>1</sub> and C<sub>2</sub>, at slightly less than 8 days. A<sub>1</sub>, B<sub>1</sub>, and C<sub>1</sub> show the third spinal segment; A<sub>2</sub>, B<sub>2</sub>, and C<sub>2</sub> show the thirteenth. All  $\times 80$ .

1, associational neuroblasts; 2, ventral commissure; 3, commissural neuroblasts; 4, dorsal white column; 5, dorsal root; 6, dorsal root efferent fibers; 7, visceral motor nucleus (preganglionic column of Terni); 8, lateral white column; 9, sensory collaterals; 10, somatic motor nucleus (lateral motor column); 11, ventral white column; 12, ventral root.

116-A<sub>1</sub> and A<sub>2</sub>). This observation has led Windle and Orr (1934) to suggest that the ventral white columns of the 5-day chick are predominantly contralateral ascending pathways. A few secondary neuroblasts send their axons into the homolateral ventral white columns. Just ventromedial to the entrance of the dorsal roots there are a small number of secondary neuroblasts which resemble the commissural elements but which send their axons into the dorsal portion of the lateral white columns (*Windle and Orr, 1934*).



The ventral and lateral white columns in the 5-day chick embryo decrease in size progressively as the cord is traced caudad. The lateral columns end at about the fourteenth cervical segment, in approximately the same region where associational cells disappear from the central gray (*Windle and Orr, 1934*). The ventral white columns continue into the caudal region of the cord. The dorsal white columns, formed by the entering dorsal root fibers, are small. No fibers ascend more than a segment or so, and the columns vary in size with the size of the entering dorsal roots (*Windle and Orr, 1934*).

**After the Fifth Day.** With the cessation of proliferation and migration of cells in the basal plate on the sixth day of the chick embryo's incubation period, the inner mantle layer disappears in that region and becomes increased in size in the alar plate (*Hamburger, 1948*). This change, together with an emergent lateral sulcus, may be seen in Fig. 114-G and H. Commissural and associational neuroblasts have increased in number; and the lateral white columns, which receive the axons of the latter cells, undergo a marked development between 5.5 and 6.5 days of incubation, as may be seen in Fig. 116-B<sub>1</sub> and B<sub>2</sub> (*Windle and Orr, 1934*). The lateral white columns are largest in the cervical region, decrease sharply in size below the brachial levels of the cord, and extend as a thin lamina as far as the base of the tail (*Windle and Orr, 1934*). The ventral and lateral white columns are predominantly ascending tracts at this stage, although a few descending fibers can be identified in them. A few collaterals have developed from the ventral, and perhaps the lateral, white columns and pass among the motor and internuncial cells of the central gray. These collaterals are most numerous in the cervical regions and are rarely found below the brachial level of the spinal cord (*Windle and Orr, 1934*). The first migration of the prospective cells of the marginal (paragriseal) nuclei (nuclei of Hofmann) from the mantle layer into the marginal layer begins in the chick during the sixth day of incubation (*Imhof, 1905*).

The dorsal white columns have expanded toward the median plane (*Hamburger, 1948*) but are still small (*Ramon, 1890b*). They can be traced forward to the level of the vagal nuclei, where they end without connecting with any longitudinal tract in the brain stem (*Windle and Orr, 1934*).

The visceromotor neuroblasts start to migrate late in the fourth day of incubation (*Levi-Montalcini, 1950*) and are now found in a position dorso-lateral to the ventral motor columns. The cervical visceral neuroblasts above the third segment emerge as the spinal accessory nerve, and many emerge with the dorsal root of the third cervical segment. Below this level visceral elements decrease rapidly in number (*Windle and Orr, 1934*).

Figures 114-I and J show the outlines of the regions of the spinal cord during the seventh day of incubation. The dorsal white column has become distinct and an intermediate region is developing in the dorsal horn (*Ham-*



burger, 1948). Ramon (1890a) stated that the cells of the developing spinal cord of the chick embryo stain best by the Golgi method between 5 and 12 days of incubation. In Fig. 115-D<sub>1</sub> are seen the cells whose axons contribute to the homolateral white columns at 7 days. These axons have a short but variable course and may contribute (1) an ascending and descending branch to the ventral white columns; (2) two ascending fibers in the ventral white columns; (3) a fiber or fibers into the lateral or the dorsal white columns; and (4) a fiber to the lateral white columns which first gives off a collateral in the central gray. Figure 115-D<sub>2</sub> shows the commissural cell types found after 7 days' incubation. The axons of these cells form the ventral (white) commissure, although there is no tendency toward commissural nuclei. The commissural cells of the dorsal horn tend to be fusiform, while the others tend to be multipolar or polygonal. The distribution of the fibers may be seen clearly in the figure.

During the chick's seventh day of incubation, collaterals from the white columns make their appearance, although a few from the ventral column can be seen on the preceding day (Windle and Orr, 1934). Figure 115-D<sub>3</sub> shows the collaterals from all three columns ramifying in the central gray. Collaterals from the dorsal white columns appear and make functional contact with secondary neuroblasts of the mantle layer. It is approximately at this time that local wing reflexes can first be evoked by mechanical stimuli (Windle and Orr, 1934). It may be noted, however, that Levi-Montalcini and Visintini (1938) observed the initial contact of sensory collaterals with secondary commissural and associational motor neuroblasts at the 5-day stage. They stated that free sensory terminations are established in the skin at the same time; thus the first reflex arc (forming the basis of the cutaneous reflex) consists of three neurones.

During the eighth day of incubation, the dorsal white columns become conspicuously larger and the central canal smaller and more oval, as may be seen in Fig. 114-K and L (Hamburger, 1948). The proliferation and migration of cells in the alar plate region is decreasing and will be terminated by the ninth day of incubation (Hamburger, 1948). The first differentiation of cells capable of storing glycogen occurs in the roof plate of the lumbosacral region of the cord during this day (Watterson, 1949). Imhof (1905), however, gave the ninth day as the first on which an actual enlargement of the alar plate region could be observed.

The lateral and mesial motor columns are well developed on the eighth day in the brachial and lumbosacral regions (Windle and Orr, 1934). The migration of the visceromotor elements dorsolaterally away from the ventral horn has been completed and these cells now occupy the position of the nucleus of Terni in the thoracolumbar and sacral regions. (Levi-Montalcini, 1950). The ventral white columns have enlarged somewhat, but the general tendency is for newly acquired fibers to displace older



fibers from the ventral white columns into the lateral white columns (Windle and Orr, 1934). Figures 116-C<sub>1</sub> and C<sub>2</sub> show the nuclear and fiber pattern of the spinal cord at this stage. The dorsal white columns are larger than previously and many more collaterals extend from them; however, only a few collaterals reach the motor neuroblasts of the basal plate to complete the simplest sensory-motor reflex mechanism (Windle and Orr, 1934).

The elaboration of collaterals from the white columns has progressed markedly by the ninth day (Ramon, 1890*b*). Their expansion is shown in Fig. 115-E<sub>1</sub>. The collaterals from the ventral white columns are the largest and ramify among the cells of the motor horn, although some may reach the base of the dorsal horn (Ramon, 1890*a*). A small group of these collaterals appear from the medial edge of the ventral white columns and arborize in the ventral commissure and in part cross to the opposite side (Ramon, 1890*a*). The collaterals from the dorsal white columns are finer, more numerous, and form small fascicles as they pass through the substantia gelatinosa (the outer layer of the dorsal sensory horn) to ramify about the cells of the dorsal horn, among the cells of the intermediate region of the central gray, and to some extent among the cells of the motor horn (Ramon, 1890*a*). A few collaterals from the dorsal white columns pass into the region to be occupied by the nucleus of Clarke's column either to arborize there or to cross into the dorsal (gray) commissure to the opposite nucleus (Ramon, 1890*a*).

Imhof (1905) stated that the elaboration of dendrites was all but completed during the period from the ninth to the thirteenth day of the chick's incubation period and that the appearance of spongioblasts was the predominant feature of the development of the spinal cord at that time. Ramon (1890*a*) observed a progressive increase in the number and complexity of collaterals and a large increase in the size of the dorsal commissure on the tenth day of incubation. Retzius (1902) described marginal cells in the 10- to 16-day chick embryo, not only in the marginal (paragriseal) region of the cord but also over its ventral surface. Kölliker (1902) noted that the marginal nuclei (nuclei of Hofmann) were completely developed by the tenth day of incubation, although Imhof (1905) believed that they continued to develop until the fourteenth day. The appearance of astrocytes (one of the two chief types of glial cells) in the spinal cord is reported to occur on the thirteenth day (Imhof, 1905).

Wenger (1950) observed that the white columns are 80 per cent homolateral associational fibers on the eleventh day and only about 20 per cent contralateral commissural pathways. The final development of ascending and descending pathways of the spinal cord, the elaboration of myelination, and the completion of terminal endings proceeds until post-hatching. The nuclear patterns at various levels in the spinal cord of the pigeon



(*Columba livia*) and the ostrich (*Struthio camelus*) have been described by Huber (1936) and Streeter (1904), respectively.

**The Visceral Motor Nuclei.** The preganglionic cells of the sympathetic (thoracolumbar autonomic) trunk ganglia arise in the spinal cord in the columns of Terni, which lie in the mediodorsal portion of the central gray from the first thoracic to the second lumbar segment. Fibers originating in these columns exit from the spinal cord in the ventral roots and synapse with the cells of the ganglia forming the sympathetic chain. Since this chain extends above and below the level of origin of preganglionic sympathetic fibers, many of the fibers extend up or down the chain for some distance. Other preganglionic fibers pass to outlying autonomic ganglia or plexuses before synapsing.

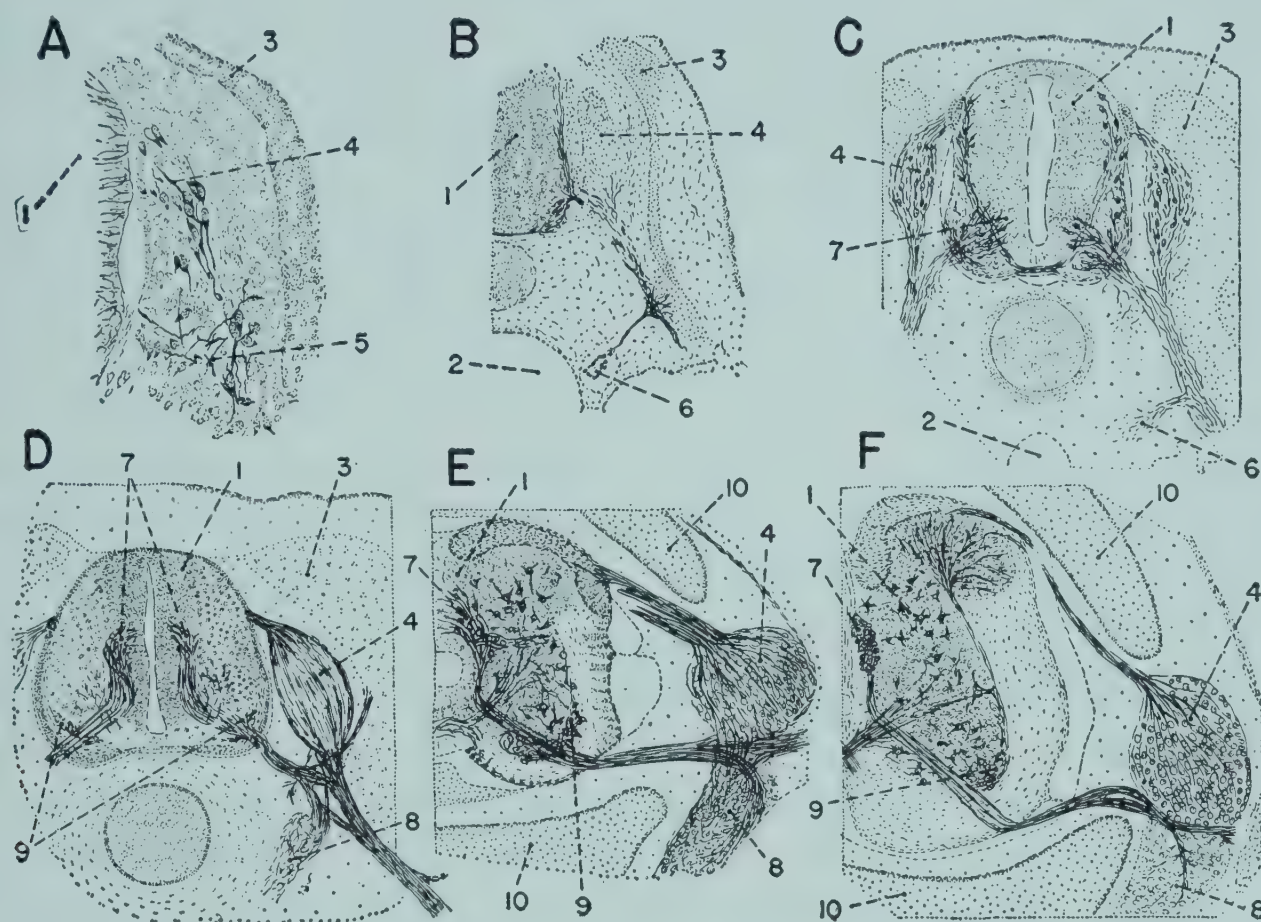
Wenger (1951) has observed that the regions of the spinal cord destined to form the preganglionic columns in the chick embryo are determined as early as the 13- to 24-somite stage. Terni (1923) first observed the preganglionic cells in the thoracolumbar cord of the chick of about 100 hours of incubation (Fig. 117-C). He described them as lying in the mantle layer adjacent to the ependyma, clearly separated from the beginning from the somatic motor column. He emphasized that the visceral motor cells originated and developed independently of the somatic motor cells. By the fifth day of incubation, the cells of the preganglionic column have migrated more dorsad (Fig. 117-D), to the site where they are to remain. The relationship of this nucleus to adjacent spinal cord structures at 9 to 10 days and at 16 days are shown in Fig. 117-E and F. The origin of the sacral preganglionic column was poorly described by Terni (1923) because of technical difficulties. He stated that it extended from the eighth to the fourteenth lumbosacral segment in the chick embryos and was located in the same position as the thoracolumbar preganglionic column.

Levi-Montalcini (1950) has re-examined the origin and development of the preganglionic system and has concluded that the nucleus of Terni does not originate independently but rather has a common origin with the somatic motor column in the ventral portion of the germinal neural epithelium. The two groups of cells are indistinguishable until 4.5 days of incubation, at which time the cells of the nucleus of Terni migrate dorso-medially; they reach their definitive position on approximately the eighth day of incubation. Prior to 4.5 days, the ventrolateral motor column is uniform throughout the cord, but after 4.5 days, regional patterns emerge. The migration described occurs in the thoracolumbar portions of the cord. A similar migration also occurs in the sacral portion of the cord from which the sacral parasympathetic outflow will develop. In cervical regions a large number of cells, presumed to be potential visceral efferent cells, degenerate during the fifth day after forming an abortive visceral system on the third and fourth days of incubation. The cells remaining



form the medial motor column. In the brachial and lumbosacral regions all the cells take part in the formation of the lateral and medial motor columns, the former of which is enlarged to innervate the extremities.

It has been observed that the preganglionic fibers are capable of forming a fairly normal pattern in the sympathetic trunk and peripheral autonomic plexuses in the absence of autonomic ganglion cells (*Müller*



**Fig. 117.** Successive stages in the development of the spinal ganglia, the sympathetic trunks, and the preganglionic columns of Terni in the spinal cord, as shown by cross sections of the spinal cord (and adjacent regions) of the chick embryo. (Redrawn with modifications A and B, after Müller and Ingvar, 1923; C, D, E, and F, after Terni, 1923.)

A, the early migration of sympathoblasts at 3 days ( $\times 100$ ); B, an early spinal ganglion and the primary sympathetic trunk in a 4-day embryo ( $\times 50$ ); C, the primary sympathetic trunk and the early migration of the preganglionic column in a 4.5-day embryo ( $\times 50$ ); D, the early secondary sympathetic trunk in a 5-day embryo ( $\times 50$ ); E, F, sections through a 9- to 10-day and a 16-day embryo ( $\times 50$  and  $\times 25$ , respectively).

1, neural tube; 2, dorsal aorta; 3, myotome; 4, spinal ganglion; 5, sympathoblasts; 6, primary sympathetic trunk; 7, preganglionic column of Terni; 8, secondary sympathetic trunk; 9, lateral motor column; 10, developing vertebra.

and Ingvar, 1923; Hammond and Yntema, 1947), although the presence of some ganglion cells is necessary in the cervical portion of the sympathetic trunk if the preganglionic fibers are to progress very far (*Yntema and Hammond, 1945*). It has been proposed that the preganglionic fibers exert some organizing influence upon the neuroblasts which form the ganglion cells of the sympathetic trunk (*Staudacher, 1940*), but enteric neurones



have been shown to develop normally in the absence of preganglionic fibers (*Campenhout, 1932; Keuning, 1948*).

**Factors Influencing the Cytoarchitectural Development of the Cord.** It is probable that the proliferation and migration of cells in the spinal cord are internally regulated and not affected by peripheral factors (*Hamburger and Keefe, 1944*). Transplantation of brachial segments of the spinal cord of embryo chicks to adjacent levels of the cord has shown that cervical, brachial, and thoracic levels of the cord are determined with respect to cell type and cell distribution prior to 2 days of incubation (*Wenger, 1951*). The differentiation of various cell types from any given portion of the neural epithelium of the chick embryo's spinal cord does not appear to depend upon any inductive influence of adjacent regions of the neural epithelium or of the mantle layer (*Wenger, 1950*), except possibly in the region of the lumbosacral enlargement where the "glycogen body" forms in the roof plate.

Many experiments have been designed to determine the factors controlling neural differentiation in the spinal cord. Williams (*1931*) observed a 13.1 per cent reduction in the size of the central gray, a 43.9 per cent reduction in the size of the white columns, and a 41.4 per cent cellular hypoplasia in the lumbosacral spinal cord of a young chicken after this region had been surgically isolated at 2.5 days of incubation. He concluded that cellular proliferation [differentiation (Ed)] in the lumbar cord of the chick embryo is influenced by stimuli from fibers growing in from other parts of the central nervous system, although the distribution and appearance of those cells which differentiate do not seem to be influenced by such stimuli. Hamburger (*1946*), however, isolated the brachial cord of 2- to 3-day chick embryos with tantalum blocks and found no difference in motor cell number between the isolated and control segments of the cord. He suggested, therefore, that longitudinally running tracts play no part in the differentiation of the lateral motor columns. Since the central gray was of normal size in the operated animals, he also inferred that proliferation, migration, and differentiation are independent of internally originating fiber systems. Wenger (*1950*) removed parts of the spinal cord from 44-hour chick embryos and examined the remaining portions at 11 days of incubation. He found evidence that removal of one lateral half of the cord does not influence the normal development of the contralateral side. Commissural fibers do not influence the development of the lateral motor columns, nor are the sensory and internuncial cells affected. Removal of the dorsal one fourth of the cord does not affect the development of the ipsilateral motor horn unless a portion of the basal plate is removed. Thus sensory reflex connections appear to have no influence upon the development of the lateral motor columns. The absence of the spinal ganglion was observed to have an effect on the development of



the dorsal horn, but the principal factor in its development was the amount of alar plate epithelium left intact. Bueker (1943), after making transplantation experiments, indicated that descending or ascending fiber connections within the cord are not necessary for the development of the lateral motor columns.

A clear peripheral effect, however, has been shown in chick embryos by limb bud extirpations which have produced a hypoplasia in the lateral motor columns of adjacent spinal cord segments (*Hamburger, 1934; Bueker, 1943, 1947; Hamburger and Keefe, 1944; Watterson and Spiroff, 1949*). Bueker (1943, 1947) has observed that limb bud extirpation in 2.5- to 3-day chick embryos results in a 75 to 90 per cent hypoplasia in the lateral motor columns by 9 to 11 days of incubation and a 90 per cent hypoplasia by 3 months post-hatching; a 50 per cent hypoplasia in the spinal ganglia at 9 to 11 days of incubation, unchanged at 3 months post-hatching; and a 27 per cent hypoplasia in the dorsal columns at 9 to 11 days of incubation, increasing to 60 per cent at 3 months post-hatching. There is only a slight hypoplasia in the intermediate region of the central gray by 3 months post-hatching. The mesial motor groups are described as being unaffected by the extirpation (*Hamburger, 1934*). Transplants of spinal cord segments with damage to the adjacent axial musculature show a hypoplasia in the mesial motor columns, however (*Bueker, 1944, 1945*).

In limb bud transplant experiments on chick embryos (*Hamburger, 1939; Bueker, 1943; Hamburger and Keefe, 1944*), hyperplasia has been observed in the lateral motor columns and spinal ganglia of segments of the cord innervating the transplant. It is thus clear that the periphery exerts an effect upon the development of cells in the spinal cord, and it is upon the process of differentiation that this effect is produced (*Hamburger, 1934, and others*). *Hamburger and Keefe (1944)* counted the total number of cells, motor and nonmotor, in affected regions of the spinal cord exhibiting hypo- or hyperplasia. They observed that the total number of cells was unchanged, but the number of motor cells (which had completed their differentiation) was less in hypoplastic segments and more in hyperplastic segments. They concluded that intrinsic factors govern the proliferation of neuroblasts and their migration, but that peripheral influences regulate the number of these cells which will differentiate into motor neurones.

**Enzyme Activity Associated with Spinal Cord Development.** Moog (1943) studied the distribution of phosphatase in the spinal cord of chick embryos from 1 to 8 days of incubation. At the end of the first day, alkaline phosphatase is present in the neural tissue in high concentrations. During the second day of incubation, the level remains high anteriorly; but near the caudal end of the developing cord, activity is reduced and almost disappears. The dorsal portion of the anterior part of the cord, on



the third day, tends to react more strongly than the ventral portion, but this difference fades in more caudal parts of the cord. During the fourth and fifth days there is a loss of alkaline phosphatase activity in the ventral horn in the region of the developing motor columns. The white matter lateral to the ventral horns reacts strongly. From the fifth day on, there is a loss of enzyme activity in an anteroposterior gradient from the dorsal portions of the cord. The pattern established is an absence of enzyme activity from the dorsal half of the cord (except for a small region at the base of the dorsal columns) and a concentration of enzyme activity in the ventral half of the cord (except for the region of the floor plate and the lateral motor column). The mesial motor column has somewhat less activity than the adjacent gray, but the difference is not as pronounced as in other areas mentioned. The white matter is very active, as is the ventral commissure. This pattern, established in the cord about on the sixth day, does not change up to the eleventh day of incubation. Acid phosphatase also appears in the nervous tissue on the first day of incubation, but seems to be less active. As cord development proceeds, acid phosphatase activity becomes concentrated in the ependymal layer. Between the fifth and seventh days, acid phosphatase activity disappears from the dorsal half of the cord, except for the area which also shows alkaline phosphatase activity, and becomes concentrated in the lateral and mesial motor columns in the ventral half of the cord. These changes also occur in an anteroposterior progression. The ventral commissure and the adjacent ependymal cells show high activity for this enzyme. This pattern remains unchanged through the eighth day of incubation. It has been suggested that both these phosphatases at first play a part in the differentiation of the neurones, and that they later are involved in the specific function of the cells in which they are concentrated.

### The Spinal Nerves

The spinal nerves are given off in pairs from either side of the spinal cord, one pair for each vertebra. Each nerve of every pair possesses a dorsal or sensory root and a ventral or motor root. Both roots contain visceral and somatic fibers. The fibers of the dorsal roots, both visceral and somatic, arise from cell bodies situated in a ganglion located on the root itself; some of the visceral fibers in the dorsal roots, however, originate in the cord. The central fibers of the sensory spinal ganglion connect with centers in the spinal cord. The somatic fibers of the ventral root originate from cell bodies in the ventral horn of the gray substance of the cord, and the visceral fibers originate from the mediodorsal portion of the gray substance.

The two nerve roots unite as they pass through the intervertebral foramina to form a very short nerve trunk which divides, upon emerging, into a dorsal or superficial branch, a ventral or deep branch, and a ramus



communicans leading to a ganglion of the paravertebral autonomic chain. The ramus communicans contains visceral sensory fibers, and, at most levels, preganglionic visceral motor fibers, both of which are part of the autonomic nervous system. At all levels, the ramus communicans contains postganglionic visceral motor fibers, originating in paravertebral autonomic ganglia of corresponding or different levels, which join the spinal nerves and are distributed in them.

### *The Spinal Nerve Roots*

The appearance of the ventral spinal nerve roots precedes that of the spinal ganglia and the dorsal nerve roots by some time. In the chick embryo, they arise during the second day of incubation, and, at rostral levels of the 2-day chick's cord, they are larger than any of the cranial nerves (*Windle and Austin, 1936*). Their origin has already been considered while discussing the development of the spinal cord.

The dorsal roots, which arise from the spinal ganglia, appear during the third day of incubation. Early in the third day (in the chick of 27 to 30 somites), the first spinal ganglia are seen, and at 60 hours of incubation (or the 32-somite stage), ganglion cells with peripheral processes and short central processes are seen at upper spinal cord levels. In the 37-somite embryo, dorsal roots are found in the rostral part of the cord (*Windle and Austin, 1936*). The first dorsal root to appear is the third cervical root, the first two being rudimentary in the chick (*Windle and Orr, 1934*). By the fifth day, dorsal roots are present in the lumbosacral region, segment 25 (*Levi-Montalcini and Levi, 1943*). Onodi (1884) has observed the presence of dorsal roots (location in the cord not stated) in the duck (*Anas platyrhynchos*) at 6 days of incubation and in the chicken at 5 days of incubation. The general appearance of the dorsal and ventral roots and their relation to each other and to the spinal ganglion and spinal cord in the chick embryo at 4.5 to 5 days of incubation are shown in Fig. 117-C and D. Clarke (1862) has described the emergent dorsal rootlets and the spinal ganglia in the 9-day chick embryo, and mentions the nucleated investment of the ganglion cells and of the nerve fibers.

In the chick embryo, the entering dorsal root fibers do not invade the mantle layer of the dorsal horn until the sixth day of incubation (*Windle and Orr, 1934*). On the seventh day (cf. Fig. 115-D<sub>3</sub>), the collaterals from the dorsal white columns invade only a short way into the dorsal horn (*Ramon, 1890b*). By the ninth day, the entering dorsal root fibers have divided into an ascending and descending branch in the dorsal white columns. The entering fiber, as well as the two branches, give rise to one to three collaterals each. These terminate principally on the cells of the dorsal horn, although some may end among the motor cells in the ventral horn or cross to the central gray matter of the opposite side via the dorsal



(gray) commissure (Ramon, 1890a). Figure 113-C shows this arrangement.

A peculiar feature of the dorsal roots of birds is the appearance of efferent fibers which arise from cells in the ventral or low intermediate regions of the central gray substance. Lenhossék (1890) described these fibers and their cells of origin in a 4-day and a 5-day chick embryo. Ramon (1890b) also observed these cells, but stated that they did not occur frequently. Figure 115-C shows the arrangement of two of these cells in the spinal cord of the chick embryo at 5 days of incubation. Windle and Orr (1934), after noting the presence of numerous efferent fibers in the dorsal root of the 6-day chick embryo, concluded that they are the avian equivalent of the lower portions of the spinal part of the eleventh cranial (spinal accessory) nerve and that they arise from the caudal extremity of the special visceral efferent nuclear column in the medulla. Another type of efferent fiber has been reported by Golub (1934) to join the dorsal roots. In the chick embryo, he found fibers arising from cells in the ventrolateral region of the central gray and emerging with the ventral roots. He stated that these fibers, immediately upon emerging from the cord, turn dorsally, join the dorsal rootlets central to the spinal ganglion, and pass to the periphery through the spinal ganglion. This author commented on the internally located fibers described by Lenhossék (1890) and Ramon (1890b) and emphasized that the fibers he had observed lay superficially.

TABLE 6  
The Number and Arrangement of Spinal Nerves in the Chicken,  
Pigeon, and Ostrich

Spinal Nerves	Chicken <sup>1</sup> ( <i>Gallus gallus</i> )	Pigeon <sup>2</sup> ( <i>Columba livia</i> )	Ostrich <sup>3</sup> ( <i>Struthio camelus</i> )
	(number)	(number)	(number)
Cervical	15	14	15
Thoracic	7	6	8
Lumbar	4	4	8
Sacral	10	9	11
Coccygeal	5 (?) *	6	9
Total	41	39	51

<sup>1</sup> Kaupp (1918), Watterson (1949); <sup>2</sup> Huber (1936); <sup>3</sup> Streeter (1904).

\* Seven coccygeal vertebrae, probably at least two without spinal nerves.

In different birds, the variation in the number and arrangement of vertebrae results in a difference in the number and subdivisions of the spinal nerves. The first cervical nerve exists between the atlas and the occipital



bone, and, like the second cervical nerve, has only a rudimentary spinal ganglion and dorsal root (Windle and Orr, 1934). The last cervical spinal nerve exists below the last cervical vertebra, and thus there is one more pair of cervical spinal nerves than there are cervical vertebrae. The remaining spinal nerves exist below, and correspond in number to, the number of vertebrae in the thoracic, lumbar, and sacral regions. Several of the most caudal coccygeal spinal nerves may be absent. Table 6 gives the subdivisions of the spinal nerves in the chicken (*Gallus gallus*), the pigeon (*Columba livia*), and the ostrich (*Struthio camelus*).

At brachial and lumbosacral levels, the spinal nerves give rise to plexuses related to the innervation of the wings and legs.

### *The Spinal Ganglia*

The early differentiation of the spinal ganglia from the neural crest in the chick embryo has been studied by Tello (1947). In the region of the prospective spinal cord, the neural crest consists at first of a continuous, unsegmented column, but its cells soon migrate into the intersomitic neural spaces and form the primordia of the spinal ganglia. The formation of segmentally arranged ganglia follows the metameric formation of the somites. Figure 118 shows sections of the neural tube of chick embryos at 37 hours, or 17 somites, 57 hours, or 33 somites, 64 hours, or 36 somites, and 80 hours, or 42 somites. Note that no ganglionic mass appears until the 36-somite stage. This agrees with the observations of Onodi (1884), who observed spinal ganglia in the 62-hour chick embryo, and of Windle and Austin (1936), who showed that no spinal ganglia are present in the 48-hour (27-somite) chick embryo and that the first ganglia appear at about 60 hours (32 somites) in the cervical region. The neural crest elements differentiate into ganglioblasts, which will become ganglion cells, and into lemnoblasts and mesectodermal cells, which will become sheath cells and the supporting cells of the ganglion (Tello, 1947).

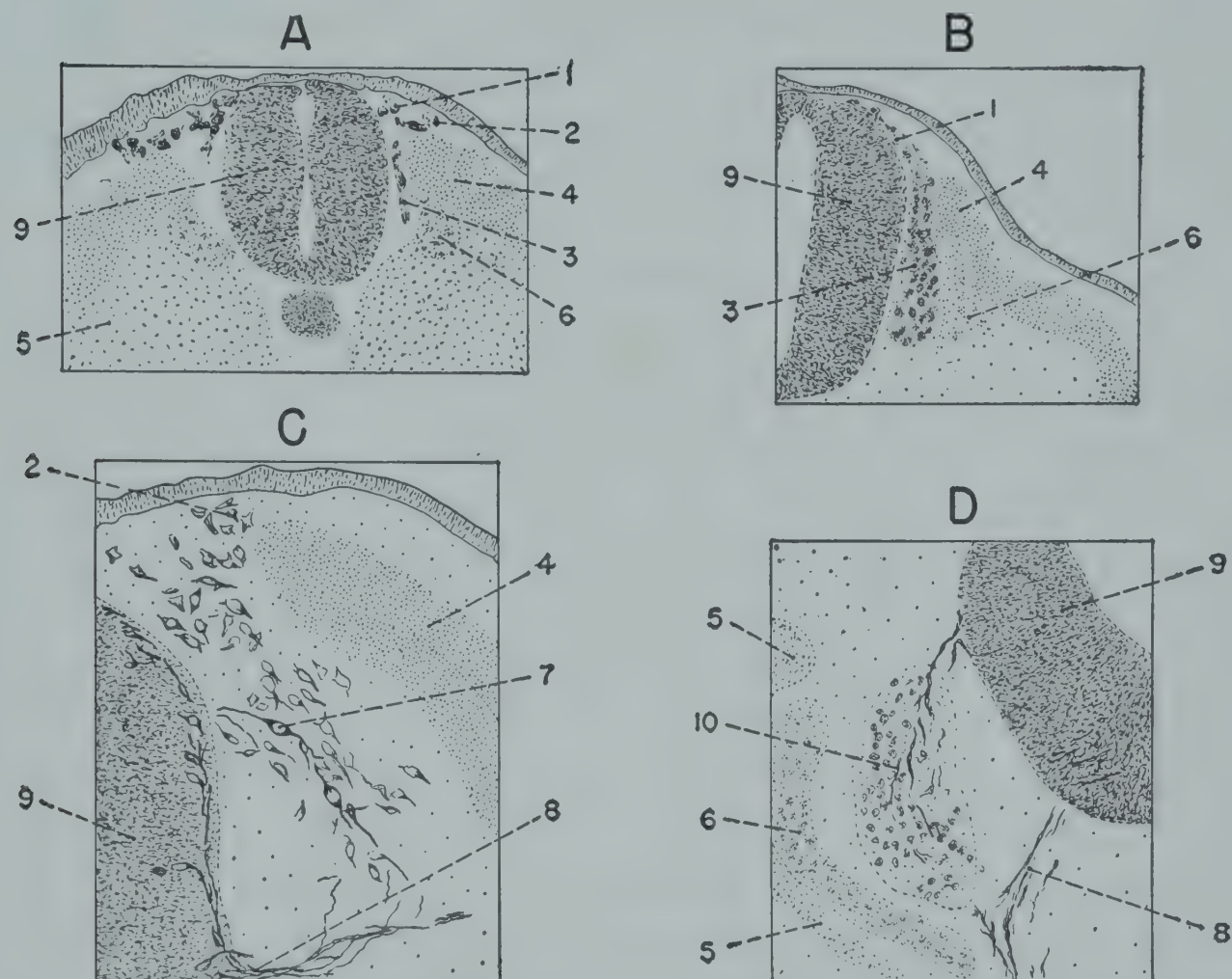
The development of the spinal ganglia has been divided into three periods by Levi-Montalcini and Levi (1943). In the chick embryo the first period extends from 2.5 days to about 8 days of incubation. During this period, the proliferation of the ganglia occurs, selective degeneration is seen in certain ganglia, and a large-celled ventrolateral group of neurones differentiates.

Proliferation begins at about 2.5 days of incubation in the chick embryo and reaches its peak, in the brachial region of the spinal cord, between the fifth and sixth days of incubation (Hamburger and Levi-Montalcini, 1949). It is completed by the eighth day (Olivo, Porta, and Barberis, 1932) or the ninth day (Hamburger and Levi-Montalcini, 1949).

Differentiation of ganglion cells also begins in the chick embryo at about 2.5 days of incubation. By 3.5 days, the number of differentiated ganglion cells is still small, and their central processes have not yet reached



the spinal cord (*Levi-Montalcini and Levi, 1943*). By the fifth day, the number of differentiated bipolar ganglion cells has increased, the dorsal root has been established, and the distal processes of the cells have reached the dermis (*Levi-Montalcini and Levi, 1943*). The early differentiation is concentrated in the ventrolateral portion of the ganglion, although, at 5 days, bipolar cells are still intermingled with undifferentiated neuroblasts,



**Fig. 118.** The development of the dorsal root ganglion from the neural crest in the chick embryo, as seen in cross sections taken at 37 to 80 hours of incubation. (Redrawn with modifications after Tello, 1947.)

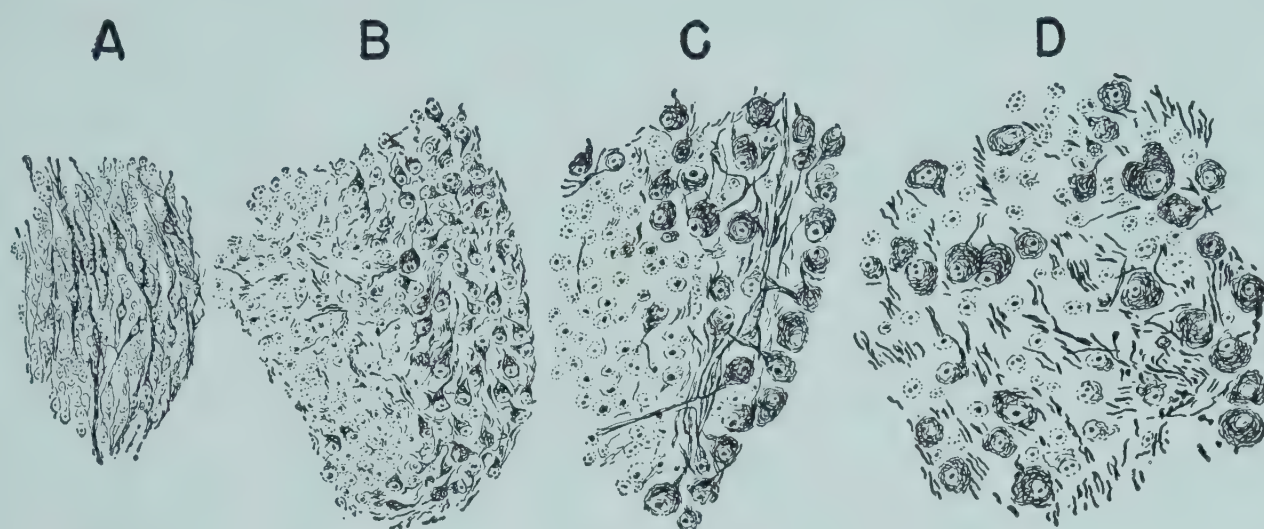
A, section of the cervical spinal cord taken between the sixth and the seventh somite of a 17-somite (37-hour) embryo ( $\times 160$ ); B, section of the lumbar spinal cord at the level of the twenty-ninth somite of a 33-somite (57-hour) embryo ( $\times 160$ ); C, section of the cervical spinal cord at the level of the seventeenth somite of a 36-somite (64-hour) embryo ( $\times 320$ ); D, section of the cervical spinal cord at the level of the fourteenth somite of a 42-somite (80-hour) embryo ( $\times 160$ ).

1, wing of the neural crest; 2, subectodermal current; 3, perineural current; 4, dermatome; 5, sclerotome; 6, myotome; 7, bipolar ganglionic neuroblast; 8, ventral root; 9, neural tube; 10, spinal ganglion.

as may be seen in Fig. 119-A. During the next few days of development these differentiated cells migrate into a ventrolateral position in the ganglion and, by the eighth day of incubation, form a group of large bipolar ganglion cells quite separate from the dorsomedial undifferentiated neuroblasts (*Levi-Montalcini and Levi, 1943*). This arrangement is seen in Fig. 119-B.



In the cervical and thoracic ganglia of the early chick embryo, degeneration of already differentiated cells was observed by Hamburger and Levi-Montalcini (1949). This degeneration reaches its peak between the fifth and sixth days of incubation and is completed by the eighth day. It affects only the large, differentiated ganglion cells in the ventrolateral part of the ganglion. Hamburger and Levi-Montalcini (1949) concluded that the large cells which survive this degeneration are tactile exteroceptive neurones, since their distal processes reach the dermis and their development coincides with the beginning of exteroceptive reflex activity in the chick embryo



**Fig. 119.** Differentiation and development of ganglion cells in a dorsal root ganglion of the chick embryo, as seen in sections of the twenty-fifth spinal ganglion by DeCastro silver stain. (Redrawn after Levi-Montalcini and Levi, 1943.)

A, at 5.5 days, bipolar ganglion cells are seen scattered among undifferentiated neuroblasts; B, at 10 days, the large differentiated bipolar ganglion cells and the undifferentiated neuroblasts are concentrated in the ventrolateral and the mediodorsal portions of the ganglion, respectively; C, at 14 days, the ventrolaterally located ganglion cells are well developed and are assuming a pseudo-unipolar appearance, and the neuroblasts in the mediodorsal portion of the ganglion have begun to differentiate; D, at 19.5 days, well-differentiated ganglion cells are seen throughout the ganglion, although many smaller cells are still in the process of differentiation. All  $\times 150$ .

(Visintini and Levi-Montalcini, 1939). Hamburger and Levi-Montalcini (1949) suggested further that the small cells in the mediodorsal part of the ganglion will differentiate into proprioceptive neurones.

The second phase of ganglion development extends from the eighth to the twelfth or fourteenth day of incubation. During this period the cells of the ventrolateral group increase in size and many of them assume a typical pseudo-unipolar appearance (Fig. 119-C). At 9 or 10 days, the smaller mediodorsal cells begin to differentiate, and the sharp increase in the number of differentiating cells at 12 days coincides with an increase in the size of the dorsal root (Levi-Montalcini and Levi, 1943).

The third phase extends from the twelfth day of incubation. During this period there is a progressive differentiation and development of the small cells (cf. Fig. 119-C). From the fifteenth day onward, two distinct groups, mediodorsal and ventrolateral, are no longer distinguishable (Levi-Montalcini and Levi, 1943).



*cini and Levi, 1943*), although the range of cell sizes has steadily increased (Fig. 119-D). Figure 120 gives a graphic indication of the frequency of ganglion-cell sizes in the fourth cervical ganglion of the chick embryo from the sixth to the twenty-first day of incubation, as shown by Olivo, Porta, and Barberis (1932).

Hamburger (1939) has observed hyperplasia in posterior brachial spinal ganglia which innervated a transplanted wing bud. Hypoplasia in spinal ganglia which innervated an extirpated limb has also been observed (*Hamburger, 1934*). Mitotic activity in spinal ganglia is reduced after limb extirpation and increased in the presence of an additional, transplanted limb.

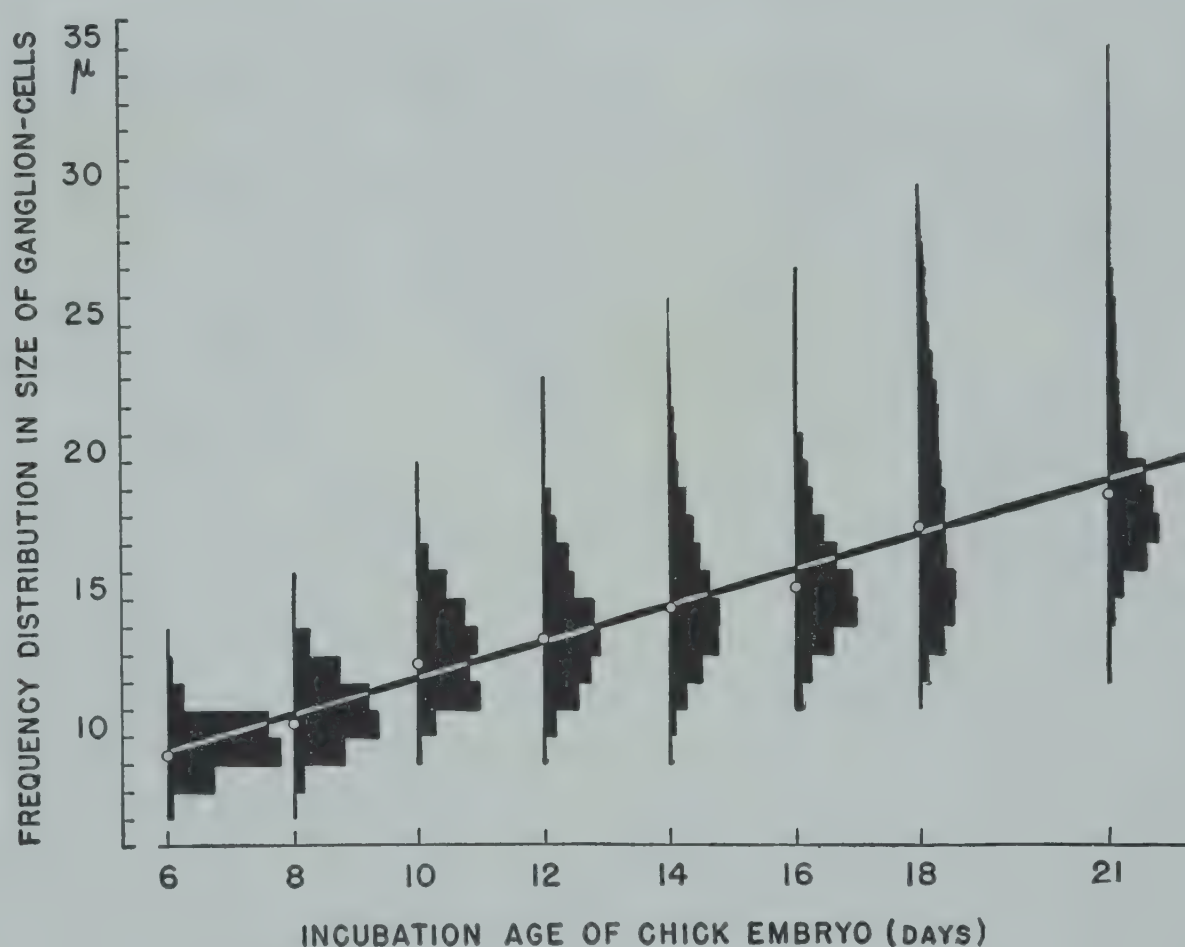


Fig. 120. The frequency of ganglion cells of different sizes in the fourth cervical spinal ganglion of the chick embryo at successive stages of incubation, as determined by counts of 1000 individual cells at each age. (Redrawn with modifications after Olivo, Porta, and Barberis, 1932, and Olivo, 1939.)

The change is approximately 20 per cent in either direction (*Hamburger and Levi-Montalcini, 1949*). Overloaded ganglia show an increase of as much as 80 per cent in the number of early differentiating neurones and a significant decrease in the amount of later degeneration. Limb extirpation, contrariwise, results in a rapid degeneration of many early differentiated cells at the fifth to sixth day of incubation. Late differentiating cells undergo atrophy. The picture in the brachial ganglia after limb extirpation is similar to that in normal cervical and thoracic ganglia (*Hamburger and Levi-Montalcini, 1949*).

Hamburger and Levi-Montalcini (1949) have suggested that a differentiation must be made between numerical and volumetric hypo- and hyper-



plasia. They also propose that the periphery exerts a controlling influence over the early proliferation of neural crest cells which form the spinal ganglia and over the initial differentiation of undifferentiated neuroblasts which have no connection of their own with the periphery. The periphery, following the first outgrowth of distal processes, continues to influence the further differentiation of cells in the spinal ganglia and provides the conditions which direct the selective degeneration of some cells and the continued growth and maintenance of others.

Burt (1943*a*, 1943*b*, 1943*c*) has investigated the effect of vitamin B<sub>1</sub> biotin, and other substances on the growth of spinal ganglion cells *in vitro*. He has concluded that thiamin chloride (in normal physiological concentrations) and biotin do not influence the growth of axons of spinal ganglion cells in culture. The growth normally observed in cultures is not affected by the introduction of intermediary carbohydrate products: pyruvic acid, lactic acid, acetaldehyde, or acetoacetic acid (Burt, 1943*b*).

### *The Spinal Nerve Plexuses*

In the brachial and lumbosacral regions of the spinal cord, there are enlargements related to the innervation of the extremities. The spinal nerves at these levels form rather complex plexuses: the brachial and the lumbosacral plexuses, respectively. Hamburger (1934) observed that a normal brachial plexus is present in the 8- to 9-day chick embryo from which the wing bud has been removed at 72 hours, provided the future site of the plexus is not disturbed at the time of operation. The peripheral distribution of nerve fibers is abnormal, however. It has been concluded that the peripheral distribution of nerve fibers and the formation of plexus patterns are determined by the tissue surrounding the neural tube (Hamburger, 1934; Wenger, 1951). Wenger (1950) has presented evidence that the sensory and motor components as well as components from different levels of the cord are independent of each other in the formation of plexuses. Bueker (1944) transplanted the lumbosacral cord of a 2.5-day embryo of the guinea hen (*Numida meleagris*) into a 2.5-day chick embryo and observed normal plexus development, a finding which indicates that the limb periphery of one genus may be sufficient to effect the normal growth of a plexus in another genus.

The nerve components of the brachial and lumbosacral plexuses in the birds have received little attention. Figure 111 shows the lumbosacral region in the chicken and the plexus in this region. Huber (1936) has described the brachial and lumbosacral plexuses in the pigeon (*Columba livia*) in some detail. Streeter (1904), quoting Fürbringer (1888) and Gadow and Selenka (1891), has treated the plexuses in the ostrich (*Struthio camelus*). Table 7 gives the spinal nerve components of both plexuses in these avian forms.



TABLE 7

Spinal Nerve Components of the Brachial and Lumbosacral Plexuses  
in the Chicken, Pigeon, and Ostrich

	Chicken * ( <i>Gallus gallus</i> )	Pigeon † ( <i>Columba livia</i> )	Ostrich ( <i>Struthio camelus</i> )
Brachial plexus	C:13, 14, 15 T:1	C:11, 12, 13, 14 T:1	T:2, 3, 4, 5, 6 ‡
Lumbosacral plexus			
a. Plexus cruralis	L:1, 2, 3,	L:1, 2, 3,	L:3, 4, 5, 6, 7 §
b. Plexus ischadicus	L:3, 4 S:1, 2, 3, 4	L:3, 4 S:1, 2, 3	L:7, 8 S:1, 2, 3, 4, 5
C = cervical	T = thoracic	L = lumbar	S = sacral

\* Watterson (1949); † Huber (1936); ‡ Fürbringer (1888), quoted by Streeter (1904); § Gadow and Selenka (1891, p. 406).

Peripheral Nerve Endings

As the nerve fiber reaches the periphery, it is confronted with a new function to perform in a new environment. In order to receive and convert external stimuli into nerve impulses, or to convert nerve impulses into stimuli for the innervated tissue, special and often complicated nerve endings are developed. These appear at the peripheral terminus of sensory neurones of the first order and at the distal end of motor nerve fibers, where motor end plates develop in the muscles to mediate muscular contraction. Many of the nerve endings of the adult bird have been described by Boeke (1925, 1926a, 1926b). Only a few observations of the embryonic development of nerve endings in birds have been made.

**Motor Nerve Endings.** Cavalié (1904) observed motor end plates in the muscles of the chick embryo of 14, 16, and 17 days of incubation. Visintini and Levi-Montalcini (1939) could find no definitive motor end plates in chick embryos of less than 9 days' incubation, but they observed a primitive neuromuscular end organ ("bouton") where motor nerve fibers contacted the myeloblasts.

Tello (1922) observed that the invasion of the embryonic chick's musculature by nerve fibers takes place as early as the fifth day of incubation. At 7 days of incubation the "exploring" fibers are easily seen (Fig. 121-A<sub>1</sub>). The "bouton-like" motor endings in contact with the developing myofibrils, as described by Visintini and Levi-Montalcini (1939), are the earliest neuromuscular organs. At 13 days, the muscle cells are better developed and the "boutons" may be clearly seen (Fig. 121-A<sub>2</sub>). The development of



definitive motor end plates does not begin until the myofibrils are differentiated (Tello, 1922). The actual formation of the motor end plate occurs in three stages: (1) a thickening along the nerve fiber (Fig. 121-A<sub>3</sub>), (2) the twisting of the thickened portion away from the fiber, and (3) the final formation of the end plate from the thickened portion of the fiber (cf. Fig.

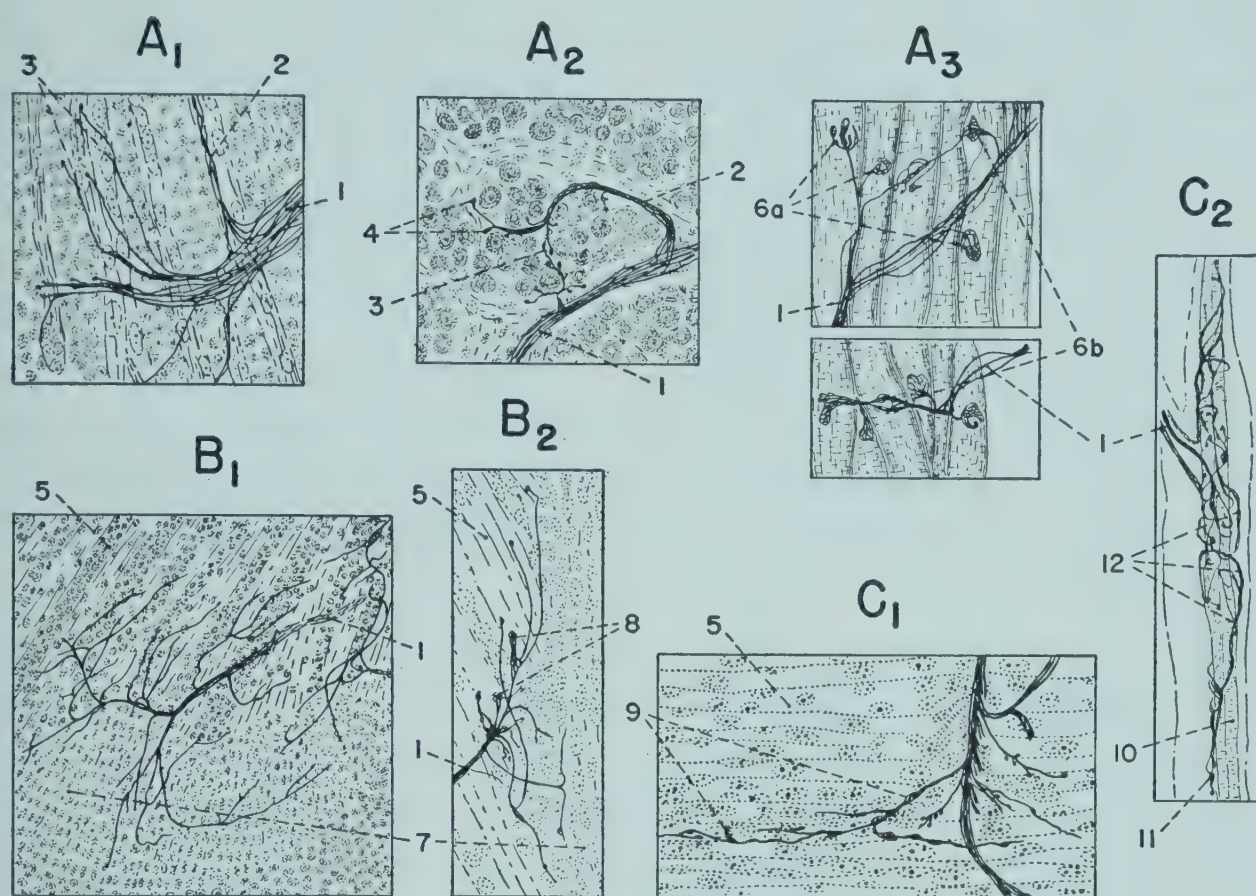


Fig. 121. The development of nerve endings in the muscles of the avian embryo. (Redrawn with modifications after Tello, 1922.)

A<sub>1</sub>, "exploring" fibers in the back musculature of the 7-day chick (*Gallus gallus*) embryo ( $\times 225$ ); A<sub>2</sub>, "exploring" fibers showing terminal "boutons" adjacent to developing myofibers in the 13-day chick embryo ( $\times 225$ ); A<sub>3</sub>, motor end plates in the tongue of a 30-day duck (*Anas platyrhynchos*) embryo ( $\times 375$ ); B<sub>1</sub>, prospective musculotendinous spindle fibers in the leg muscle of the 9-day chick embryo ( $\times 75$ ); B<sub>2</sub>, the formation of the musculotendinous spindle in the 12-day chick embryo ( $\times 225$ ); C<sub>1</sub>, the formation of the muscle spindle in the 11-day chick embryo ( $\times 225$ ); C<sub>2</sub>, the muscle spindle in the 14-day chick embryo ( $\times 225$ ).

1, nerve entering muscle; 2, branch of nerve; 3, "exploring" fiber; 4, terminal "bouton"; 5, developing myofibers; 6a, motor end plates of the terminal type in three stages of development; 6b, motor end plates of the collateral type in two stages of development; 7, tendon; 8, prospective musculotendinous spindle; 9, prospective muscle spindle; 10, myofibrils; 11, motor nerve to motor end plate in muscle; 12, spiral muscle spindle.

121-A<sub>3</sub>). A single motor fiber, usually large and myelinated, divides several times before reaching its end organ, a muscle fiber. When the axon makes contact with a muscle fiber, the myelin is lost, the neurilemma sheath (of Schwann) of the axon fuses with the sarcolemma (muscle sheath), and the axon itself, now quite fine, penetrates the substance of the muscle, where it forms the greatly enlarged end plate.



**Sensory Nerve Endings.** The sensory endings within the muscles are of several types. Tello (1922) has described the development of the musculotendinous apparatus and of the neuromuscular spindles in the chick. At about 9 days of incubation a few sensory fibers in the developing muscles may be seen to arborize in the region of the junction of the prospective muscle and tendon substance (Fig. 121-B<sub>1</sub>). By the twelfth day of incubation, the musculotendinous spindles begin to form (Fig. 121-B<sub>2</sub>) and the nerve fiber branching has become markedly more complex. The neuromuscular spindles develop slightly later than the musculotendinous spindles (Tello, 1922). At 11 days, sensory fibers may be identified, branching and coursing in the long axis of the primitive muscle cells. Small swellings can be seen along these nerve fiber branches (Fig. 121-C<sub>1</sub>). During the eleventh to thirteenth days, the muscle fibers are proliferating, and the sensory nerve fibers continue to branch, thus providing additional fibers to the muscles. By the fourteenth day, the addition of nerve fiber branches has produced a primitive muscle spindle (Fig. 121-C<sub>2</sub>). During the remaining period of embryonic development the structure is refined and completed.

Cutaneous sensory endings have been described by Boeke (1925, 1926a, 1926b), Heringa (1917, 1920), Szymonowicz (1897), and Tello (1922). The development of the Pacinian corpuscle (mediating the modality of pressure) has been described by Tello (1922), and that of the Grandry and Herbst corpuscles by Szymonowicz (1897) and Heringa (1917). All are encapsulated in many layers of connective tissue and are well suited for mediating pressure sensibility.

As early as the chick's seventh day of incubation, nerve fibers may be seen passing into the region of the developing knee joint (Fig. 122-A<sub>1</sub>). These fibers branch profusely, and the early stages of the Pacinian corpuscle may be seen by the eleventh or twelfth day (Fig. 122-A<sub>2</sub>). At 15 days, the corpuscle is well developed, although the characteristic lamellae are poorly differentiated and still few in number (Fig. 122-A<sub>3</sub>).

Szymonowicz (1897) was first able to identify the developing Herbst corpuscles in the duck (*Anas platyrhynchos*) embryo incubated about 21 days. At 24 days, the fine nerve fiber penetrates a developing capsule and lies adjacent to two or three columns of darkly staining cells (Fig. 122-B). From that time until well after hatching the concentric lamellae of the capsule are successively formed and the nerve terminus itself is refined (Szymonowicz, 1897). The appearance of these endings just after hatching is shown in Fig. 122-D.

The Grandry corpuscle, a tactile ending, was first described in the soft leathery skin over the bill of the duck (*Anas platyrhynchos*) and goose (*Anser anser*), but has since been observed in numerous waterfowl and in many other birds. The definitive corpuscle is composed of several thick circular cells enclosed in a connective tissue capsule. Between these tactile



cells is the tactile disk in which the entering nerve fiber terminates. The morphology of the definitive ending has been discussed extensively by Boeke (1926b). The development of this nerve ending has been described by Szymonowicz (1897) and by Heringa (1917). During the period from

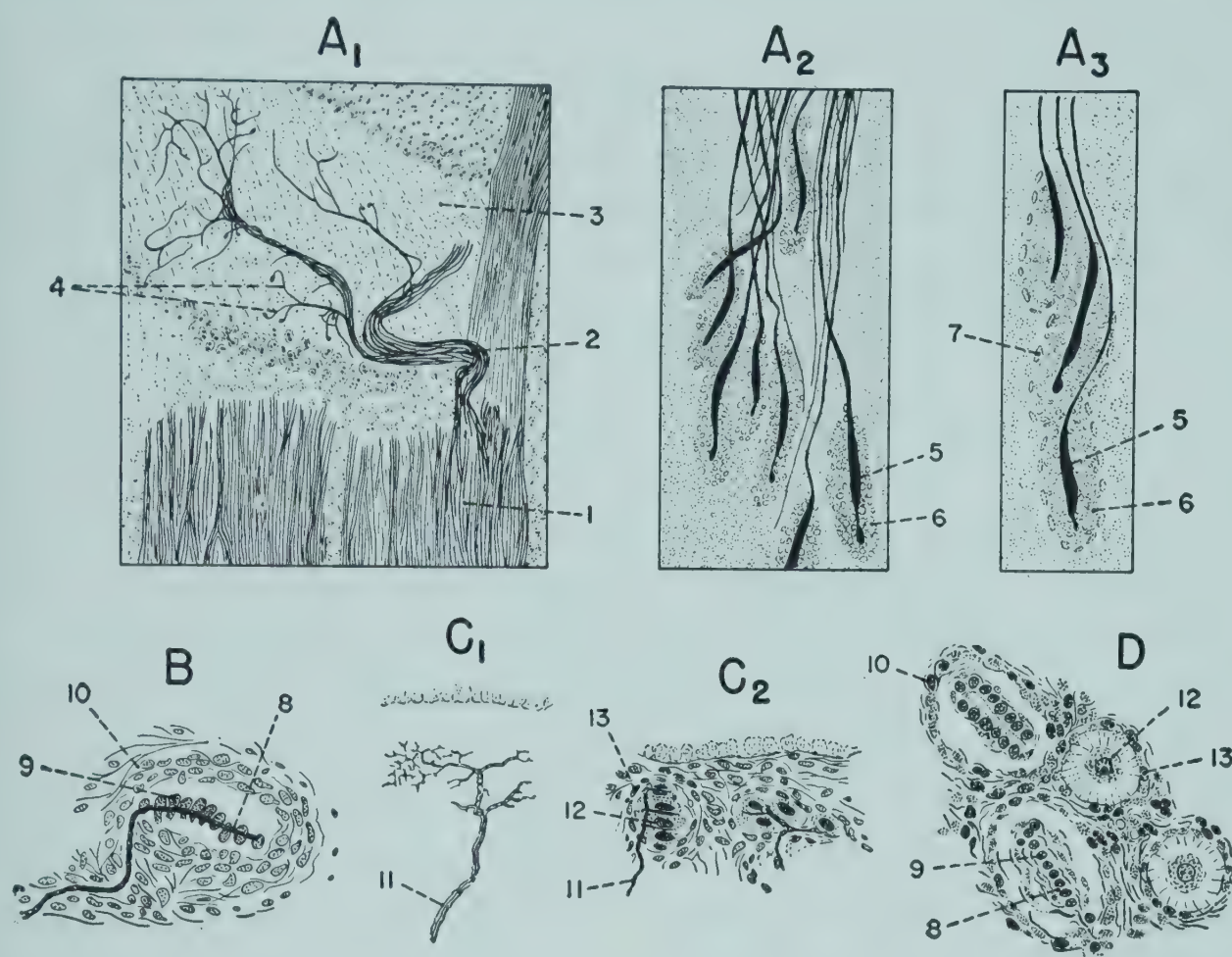


Fig. 122. The development of cutaneous nerve endings in the avian embryo. (Redrawn with modifications A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, after Tello, 1922; B, C<sub>1</sub>, C<sub>2</sub>, D, after Szymonowicz, 1897.)

A<sub>1</sub>, branches from the nervus ischiadicus femoralis posterioris to the knee joint of a 7-day chick embryo ( $\times 125$ ); A<sub>2</sub>, Pacinian corpuscles in the membrana interossea peroneo-tibialis of a 13-day chick embryo ( $\times 125$ ); A<sub>3</sub>, Pacinian corpuscles in the same membrane in a 15-day chick embryo ( $\times 125$ ); B, corpuscle of Herbst from a 24-day duck (*Anas platyrhynchos*) embryo ( $\times 200$ ); C<sub>1</sub>, branching of a cutaneous nerve in the subcutis of a 21-day duck embryo ( $\times 200$ ); C<sub>2</sub>, corpuscle of Grandry in the subcutis of a 24-day duck embryo ( $\times 200$ ); D, tactile corpuscles of Grandry and pressure-receptive corpuscles of Herbst in the duck just after hatching ( $\times 200$ ).

1, nervus ischiadicus femoralis posterioris; 2, ramus articulatus; 3, knee joint capsule; 4, terminal expansions which will form the Pacinian corpuscle; 5, nerve fiber to the developing Pacinian corpuscle; 6, connective tissue shell; 7, first connective tissue capsular lamella; 8, sensory nerve to a Herbst corpuscle; 9, tactile cells of Herbst's corpuscle; 10, forming capsular lamella of Herbst's corpuscle; 11, branching sensory fibers in region of the epidermis; 12, tactile cells of Grandry's corpuscle; 13, connective tissue capsule of Grandry's corpuscle.

16 to 20 days (Heringa 1917) or from 18 to 20 days (Szymonowicz, 1897), the cutaneous nerves reach the epidermal region of the developing duck (*Anas platyrhynchos*) embryo and a subcutaneous plexus is established. On the twentieth (Heringa, 1917) or twenty-first (Szymonowicz, 1897)



day, these nerves begin to branch extensively, the branches lying horizontally in the subcutis (Fig. 122-C<sub>1</sub>). At 24 days, the branches are seen lying between the developing tactile cells, and the connective tissue capsule has begun to appear (Fig. 122-C<sub>2</sub>). Just after hatching (Fig. 122-D), the Grandry corpuscle has assumed most of its definitive characteristics (Szymonowicz, 1897).

The tactile cells of Grandry's corpuscles and the pressure-receptive cells of Herbst's corpuscles are derived from the mesenchyme, and the differentiation of these cells is under the influence of the sensory nerve fibers which form the functional portion of the nerve ending (Szymonowicz, 1897).

### The Cranial Nerves

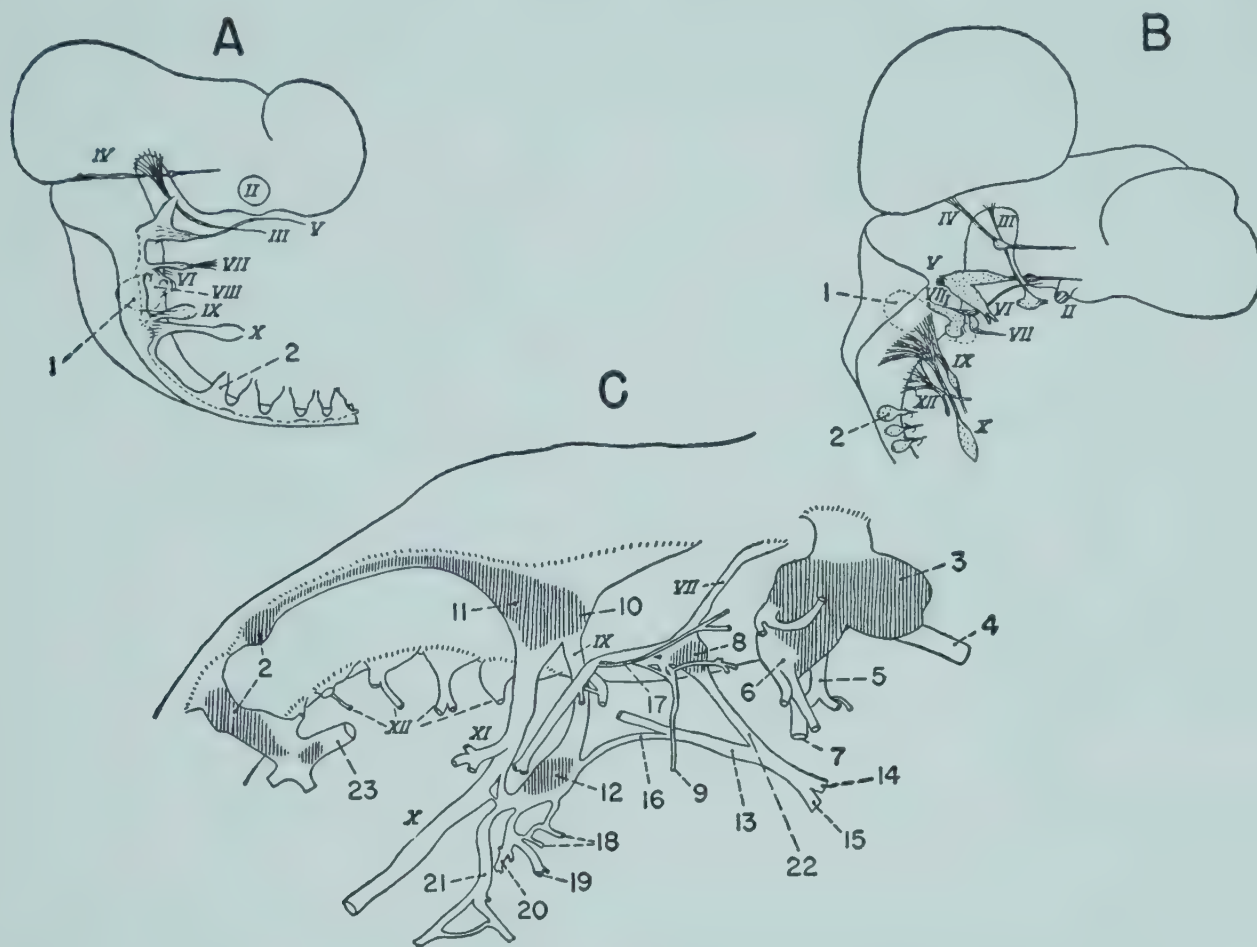
The twelve pairs of cranial nerves cannot be analyzed with the facility with which one analyzes the spinal nerves. Many attempts have been made to fit the cranial nerves into a simple segmental scheme homologous to the obvious pattern of the spinal nerves, but it is not possible to sort out the origins of the various cranial nerves with any certainty. Functionally, they are heterogeneous, and resemble the spinal nerves as little as they resemble them morphologically (Fig. 123). Some cranial nerves are purely motor, some are purely sensory, some are both. A close scrutiny of their function and the distribution of their fibers reveals that they contain four special classes of nervous elements as well as the four general classes found in the spinal nerve roots.

It will be remembered that the dorsal and ventral roots of the spinal nerves contain, respectively, general sensory and motor elements, both somatic and visceral. General somatic sensory fibers are found in the trigeminal (fifth cranial) nerve and perhaps in the three nerves (the third, fourth, and sixth) supplying the ocular musculature. The eleventh cranial (or spinal accessory) nerve contains general somatic motor elements. Both general visceral sensory and motor fibers are present in the seventh, ninth, and tenth cranial nerves, and general visceral motor fibers partially comprise the third cranial nerve as well. Cranial nerves containing general visceral elements are part of the cerebrospinal division of the autonomic nervous system.

To these classes of nerve fiber are added the special somatic and visceral elements, motor and sensory. The third, fourth, and sixth cranial nerves, innervating the ocular musculature, contain special somatic motor fibers, as does also the twelfth cranial nerve. Special somatic sensory fibers are limited to the eighth cranial nerve, supplying the cochlea and the vestibular apparatus, and to the first (olfactory) and second (optic) nerves (although the optic nerve may also be considered an integral part of the brain). The cranial nerves innervating those striated muscles of the head and neck which are derived from the mesoderm of the visceral arches possess special



visceral motor fibers. These are the fifth, seventh, ninth, tenth, and eleventh cranial nerves (the first three are proper to the first, second, and third visceral arches, respectively, and the last two are proper to the lower visceral arches). Special visceral sensory fibers are found in the first (olfactory) cranial nerve and in the seventh, ninth, and tenth cranial nerves.



**Fig. 123.** Three stages in the development of some of the cranial nerves of the chick, shown diagrammatically. (Redrawn with modifications A and B, after Bok, 1915; C, after Yntema, 1944.)

A, the cranial nerves at the end of the fourth day of incubation ( $\times 6.5$ ); B, the same at the end of the fifth day ( $\times 6.5$ ); C, some of the cranial nerves in the region of the medulla oblongata on the eighth day of incubation ( $\times 16$ ).

II, optic nerve; III, oculomotor nerve; IV, trochlear nerve; V, trigeminal nerve; VI, abducens nerve; VII, facial nerve; VIII, acoustic nerve; IX, glossopharyngeal nerve; X, vagus nerve; XI, spinal accessory nerve; XII, hypoglossal nerve; 1, auditory vesicle; 2, spinal ganglion; 3, sensory ganglion of V; 4, ophthalmic nerve; 5, motor portion of V; 6, maxillary nerve; 7, mandibular nerve; 8, geniculate ganglion; 9, chorda tympani; 10, superior ganglion; 11, jugular ganglion; 12, petrosal ganglion; 13, sphenopalatine nerve; 14, pterygopalatine nerve; 15, nasopalatine nerve; 16, tympanic nerve; 17, paratympanic nerve; 18, palatine nerve; 19, lingual nerve; 20, pharyngeal nerve (branch of IX); 21, pharyngeal nerve (branch of X); 22, greater superficial petrosal nerve; 23, sympathetic trunk.

As previously mentioned nuclei of the cranial nerves are found in the six longitudinal zones of the brain stem, and cranial nerve development is thus inextricably associated with the development of fiber tracts and nuclei in the brain. The motor components of the cranial nerve nuclei arise from a medially lying group of neuroblasts, and at 5 days of incubation (in the chick) those destined to make up the visceral motor column migrate



laterally (Fig. 124-A). By 7 days (Fig. 124-B), all of the motor nuclei have attained their definitive positions (Bok, 1915). The medial motor column contains the motor nuclei of the third, fourth, sixth, and twelfth cranial nerves and is continuous with the ventral motor column of the spinal cord. In the intermediate motor column are found the special visceral motor components of the fifth, seventh, ninth, tenth, and eleventh cranial nerves; and the lateral motor column includes the general visceral components of the tenth cranial nerve.

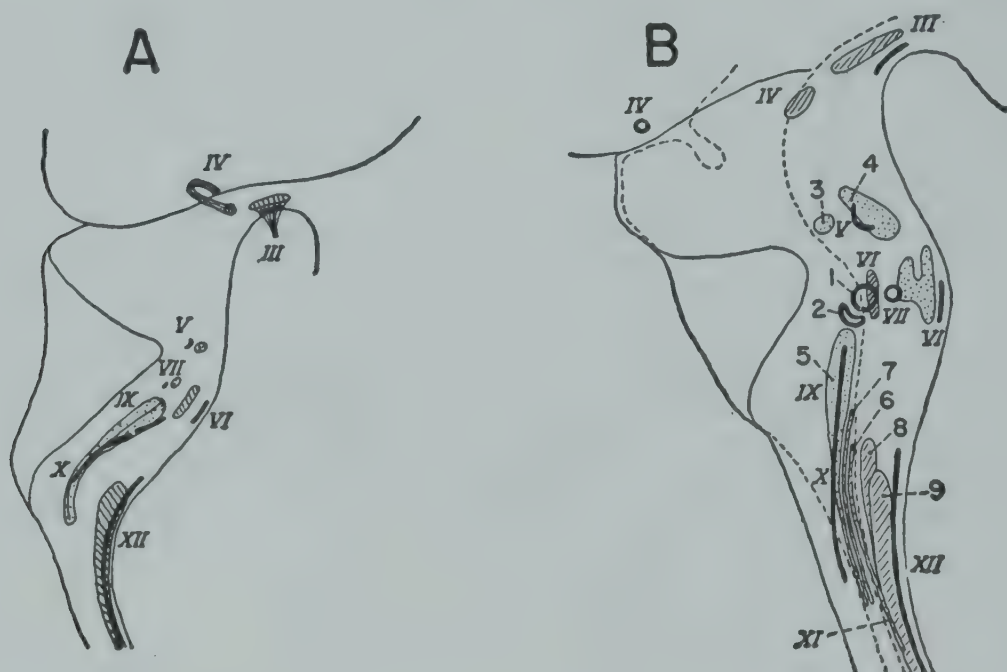


Fig. 124. The size, shape, and position of the motor nuclei of the cranial nerves in the chick embryo, shown diagrammatically. (Redrawn with modifications after Bok, 1915.)

A, after 5 days of incubation; B, after 7 days. Stippling indicates the lateral nuclei, oblique lines the medial nuclei. Heavy lines show the points of exit of the nerves, which are designated by Roman numerals. Both  $\times 10$ .

1, exit of vestibular division of VIII; 2, exit of cochlear division of VIII; 3, dorsal nucleus of V; 4, ventral nucleus of V; 5, dorsal nucleus of X; 6, ventral nucleus of X; 7, nucleus intermedius; 8, dorsal nucleus of XII; 9, ventral nucleus of XII.

Lateral to the motor columns lie the sensory columns. The most medial is the visceral sensory column (both general and special), containing the seventh, ninth, and tenth nerves, whose fibers form the tractus solitarius. The general somatic sensory column is represented by the sensory portion of the trigeminal (fifth cranial nerve) complex, and of the ninth and tenth nerves, and is intermediate between the visceral column and the special somatic sensory column associated with the vestibule and the cochlea.

Ganglia are associated with several of the cranial nerves (cf. Fig. 123). Neural crest elements are probably involved in the development of these ganglia, but in what way or to what extent is not entirely clear. There is evidence that cells which bud off the inner surface of the ectoderm in certain areas (placodes) contribute elements to the ganglia of the mixed (V, VII, IX, and X) cranial nerves (Beard, 1888b; Disse, 1897; Campenhout,



1937a, 1943; Tello, 1946; Ortmann, 1948), and this contribution is probably of considerable magnitude. It has been suggested (*Campenhout, 1937c*) that many cells of neural crest origin degenerate in the rhombencephalic region and, in so doing, release substances which induce the budding of cells from the ectoderm.

### *The Olfactory Nerves*

The olfactory (cranial I) nerves are special visceral sensory and are small in birds, which have an extremely microsomatic forebrain. The nerves originate bilaterally from the epithelium of the olfactory pits and grow centripetally from the pits to the olfactory lobes at the anterior extremities of the cerebral hemispheres.

It is of historical interest that early investigations led to the opposite conclusion; that is, that the olfactory nerve grew centrifugally (from the brain outward). Furthermore, its origin from the most cranial portion of the neural crest was assumed (*Marshall, 1878, 1879*). The nerve was supposedly composed of nonfibrillar elements until the seventh incubation day, when fibers were first observed. Also, a ganglion was said to be present at either end of the nerve (*Marshall, 1879*).

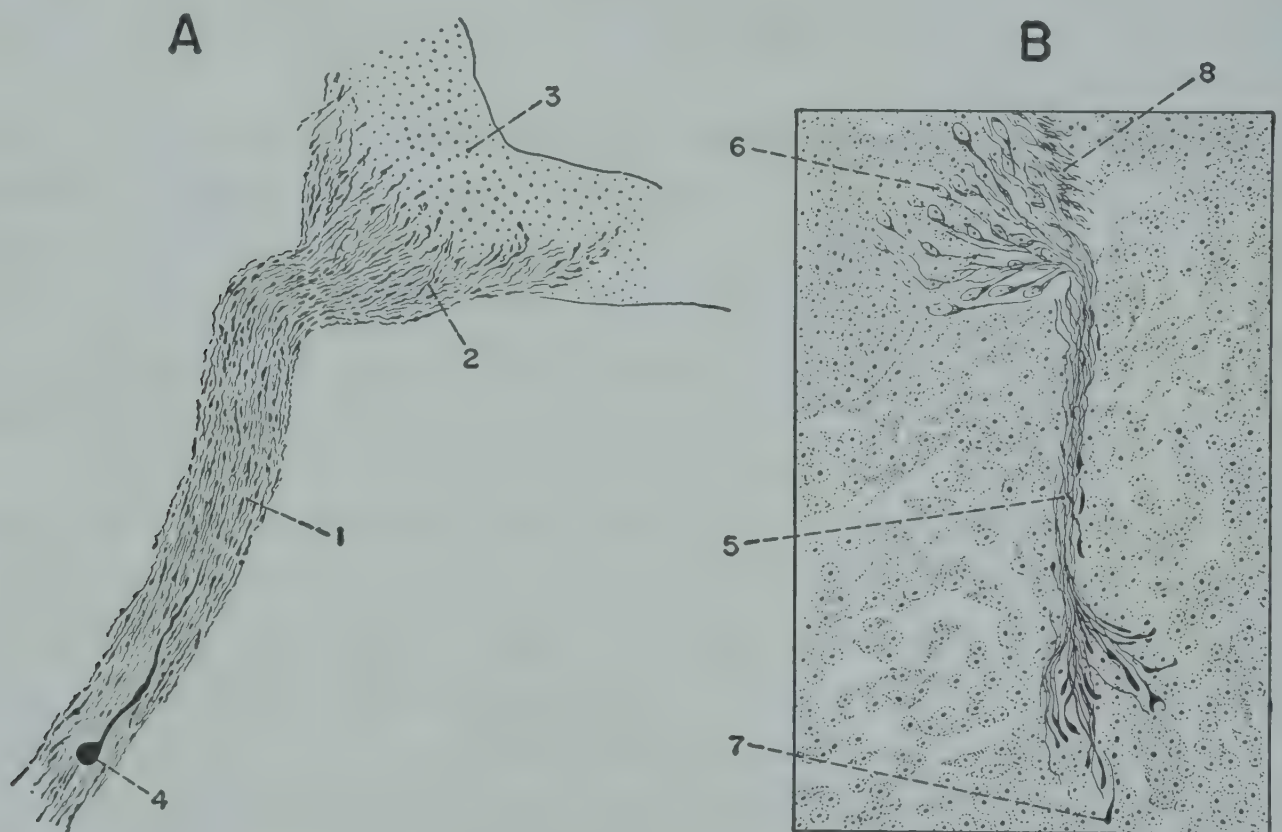
The actual manner in which the olfactory nerve is established was first suspected by Kölliker (1890). Although he was unable to find the olfactory nerve in the chick on the third incubation day, he identified it on the fourth day as an epithelial proliferation from the olfactory pit, and was able to trace it as far as the brain. He found it had fused into the ventral wall of the telencephalon on the fifth day. He observed both fibers and nuclei in the growing nerve and regarded each fiber as the product of many cells.

More detailed studies by Disse (1897), Tello (1923), and *Campenhout (1937a)* have revealed that the inception of the olfactory nerve can be detected very shortly after the appearance of the olfactory placode. At the end of the chick's second incubation day or the beginning of the third (when about 30 somites are present), epithelial cells start to bud off from the medial or inner face of the placode and to migrate toward the brain (*Campenhout, 1937a*). Some of these cells come into contact with the ventral wall of the telencephalon by the sixty-fifth hour at which time a cellular strand of considerable size has been established.

Disse (1897) showed that clearly differentiated neuroblasts can be found between the epithelial cells of the chick's olfactory placode during the course of the third day of incubation. At the end of this day, or early in the fourth, fibers become apparent in the nerve. They are most numerous at the placodal end. The medial end of the strand of cells spreads out over the ventral surface of the telencephalon, and it is possible that some penetration of fibers into the central nervous system occurs at 72 hours (*Campenhout, 1937a*).



It is apparent, at this time and during the next 2 days, that most of the fibers issue from neuroblasts situated within the olfactory placode. These neuroblasts each send out a second short process which extends in the opposite direction and terminates on the distal surface of the olfactory epithelium (that is, on the inner surface of the olfactory pit). Some of the fibers in the nerve, however, originate from bipolar cells which are lodged within the course of the nerve itself, having previously migrated out of the olfactory placode. The distal fibers of some bipolar cells in the nerve extend to the inner surface of the olfactory pit.



**Fig. 125.** Early stages in the development of the olfactory and oculomotor nerves in the chick embryo. (Redrawn with modifications A, after Disse, 1897; B, after Tello, 1923.)

A, longitudinal section through the olfactory nerve of the 8-day chick embryo ( $\times 60$ ); B, section through the oculomotor nerve and its nucleus in the 52- to 55-hour chick embryo ( $\times 250$ ).

1, olfactory nerve; 2, central end of olfactory nerve; 3, olfactory bulb of telencephalon; 4, unipolar nerve cell; 5, oculomotor nerve; 6, nucleus of oculomotor nerve; 7, swelling (cone of growth) at end of nerve fiber; 8, medial longitudinal fasciculus.

During the course of the fourth day, as fibrillization of the nerve continually increases, it becomes apparent that the epithelioid cells forming the bulk of the cellular strand are starting to differentiate as supporting cells. Some cells with fusiform nuclei have elongated, and have assumed the characteristics of nerve sheath cells (cells of Schwann). The remainder are still epithelioid or in a transitional state (*Campenhout, 1937a*).

Penetration of the fibers of the olfactory nerve into the brain takes place at 78 to 80 hours, according to *Campenhout (1937a)*; at 96 hours, according to *Windle and Austin (1936)*; at the end of the fifth day, according to



Tello (1923); and during the sixth day, according to Disse (1897). Tello (1923) stated that there is no neurofibrillar differentiation in the olfactory region of the telencephalon on the sixth day, and that bipolar and multipolar neurones appear in the developing olfactory lobes on the seventh day. He then added that contact between the fibrils of the olfactory nerves and those in the olfactory lobes is not made until the olfactory glomeruli develop. This account is somewhat at variance with that of Windle and Austin (1936), who found that the primitive olfactory pathway was completed before any other. They observed that olfacto-hypothalamic or -subthalamic tracts were initiated in the 32-somite (60-hour) chick by the development of a few fibrillated neuroblasts in that portion of the telencephalon adjacent to the olfactory placodes. In the 4-day chick the entering fibers of the olfactory nerves were found to terminate among secondary olfactory neuroblasts in the telencephalon, and the olfactory lobes of the 5-day chick contained many fibers.

Figure 125-A shows the central termination of the chick embryo's olfactory nerve as it appears on the eighth day of incubation.

### *The Optic Nerves*

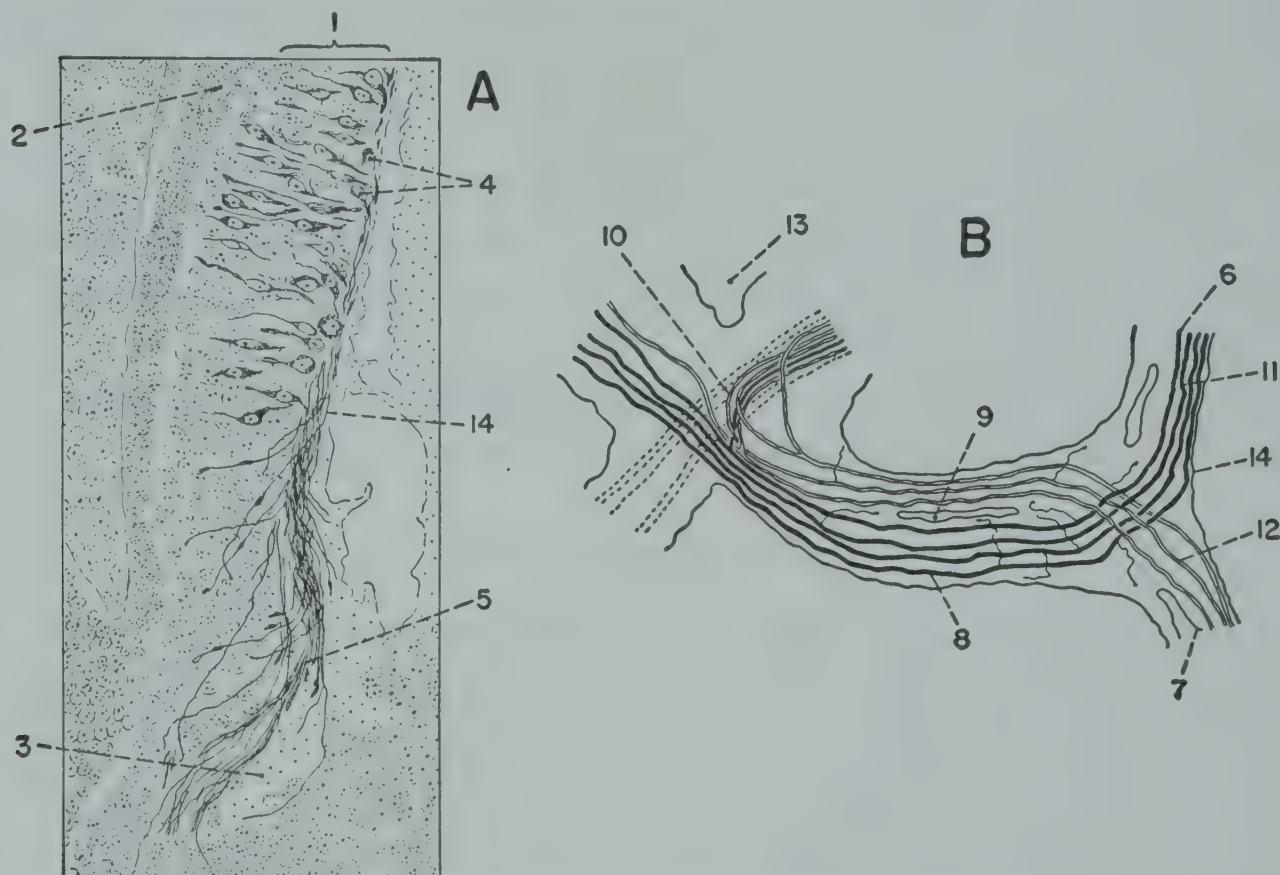
The optic (cranial II) nerves, like the olfactory nerves, are sensory and develop centripetally, but they are very large and can be considered as a special fiber tract of the brain stem proper. The optic nerves originate by differentiation of retinal cells which are derived from the neural tube. As the optic nerves leave the retina they converge toward the ventral midregion of the diencephalon and cross each other in a massive decussation, the optic chiasma. Beyond this point the fibers are usually referred to as the optic tracts. The optic tracts terminate in part in the lateral geniculate body, but principally in the optic lobes, over which they spread as a fibrous envelope. The majority of the fibers terminate in the side of the brain contralateral to the retina of origin.

It was once thought that the optic nerve developed *in situ* by differentiation of cells of the primitive optic stalks. His (1868, *p.* 131) was the first to state that this was not the case, although he simultaneously embraced the erroneous opinion (which he later reversed) that the fibers of the optic nerve grow from the brain toward the retina. Investigations on various animals eventually revealed the true origin of the optic nerve, and Assheton (1892) demonstrated the progressive growth of the nerve from the retina toward the brain in 4- and 5-day chick embryos. It appears, however, that the course of the optic nerve is not determined by any neurotropic attraction exerted by the brain, for the growing fibers are completely deflected when a barrier is placed across their normal pathway (Ferreira-Berrutti, 1951).

The initial differentiation of neuroblasts in the developing retina of the



chick apparently occurs at 46 to 48 hours of incubation (that is, in the 25- to 27-somite embryo). It was in a chick of this age that Tello (1923) noted about 18 primary bipolar neuroblasts, each with very delicate neurofibrils, directly in the center of the prospective sensory layer of the optic cup, that is, in the layer which originally formed the distal, or lateral, wall of the primary optic vesicle. In the 30- to 32-somite embryo (Tello, 1923; Windle and Austin, 1936; Berthoud, 1943) the bipolar



**Fig. 126.** The origin and course of the optic nerve in the chick embryo. (Redrawn with modifications after Tello, 1923; B, after Berthoud, 1943.)

A, section through the junction of the optic stalk and the optic vesicle of the 72-hour (36-somite) chick embryo ( $\times 200$ ); B, diagram showing the course of the optic nerve fibers after leaving the retina of the 5-day chick embryo (broken lines represent fibers of the optic nerve of the opposite side).

1, sensory layer of retina; 2, pigmented layer of retina; 3, chorioid fissure; 4, cells of ganglionic layer; 5, fibers leaving retina and entering optic stalk; 6, dorsal portion of retina; 7, ventral portion of retina; 8, optic nerve; 9, lumen of optic stalk; 10, optic chiasma; 11, fibers from dorsal retinal field; 12, fibers from ventral retinal field; 13, third ventricle; 14, internal surface of retina.

neuroblasts are much more numerous. Their axons course on the surface of the retina in the direction of the chorioid fissure, but they do not reach the optic stalk. Shortly afterward, when the embryo possesses 35 to 37 somites, retinal fibers enter the optic stalk and proceed some distance toward the site of the future optic chiasma (Fig. 126-A). The fibers follow the inferior surface of the optic stalk, and their number decreases with greater distance from the retina. A few fibers penetrate into the retina instead of joining those on the surface (Ramon, 1905; Tello, 1923). At



this stage the differentiation of a few neuroblasts can be detected in the more ventral portion of the retina, which is divided from the dorsal portion by the point of origin of the optic stalk (*Berthoud, 1943*).

The fibers of the optic nerves attain, and cross through, the optic chiasma for the first time at the beginning of the fourth day (*Tello, 1923; Windle and Austin, 1936*), but only a few extend as far as the superior surface of the diencephalon (*Tello, 1923*). By the fifth day, the lumen of each optic stalk has disappeared at the two extremities, although it still exists in the median portion (*Berthoud, 1943*). On this day, the retinal fibers first reach the surface of the optic lobes (*Tello, 1923*). The optic nerve becomes solid on the sixth day in the embryo of the zebra parakeet, *Melopsittacus undulatus* (*Abraham, 1901*).

At the beginning of the fifth day neurofibrillar development in the ventral portion of the retina has become marked (*Berthoud, 1943*). By this time, differentiation has progressed to the equator of the retina, and, in consequence, all degrees of neuroblast development can be observed from the center of the retina, where it is most advanced, to the equator (*Tello, 1923*). According to *Berthoud (1941, 1943)*, the fibers derived from the ventral portion of the retina cross over those from the dorsal portion at the point of emergence from the retina and course along the dorsal side of the optic stalk. At the optic chiasma most of the fibers of ventral retinal origin, and a few of dorsal origin, fail to cross, but instead enter the optic tract on the same side (*Fig. 126-B*). Partial decussation of the optic nerve in the chick embryo has also been demonstrated by *Ferreira-Berrutti (1951)*.

On the seventh day large numbers of fibers of retinal origin may be seen coursing over the surface of the optic lobes.

### *The Oculomotor Nerves*

The oculomotor (cranial III) nerves are motor nerves with both general visceral and special somatic elements. The latter components innervate the homolateral ocular muscles, with the exception of the superior oblique and external rectus muscles. The general visceral components innervate the ciliary muscles via the ciliary ganglion.

There is general agreement that the oculomotor nerves originate by centrifugal growth from the ventral aspect of the midbrain, although *Marshall (1878)* suggested that they are derived from neural crest cells which have migrated to a ventral position. On the other hand, there is still doubt regarding the origin of the ciliary ganglia.

In the 48-hour (27-somite) chick oculomotor nerve fibers can be seen passing ventrally from a small nucleus of bipolar and monopolar cells situated in the base of the mesencephalon, on either side (*Tello, 1923; Windle and Austin, 1936; Campenhout, 1937a*). The nucleus of origin



of the oculomotor nerve, almost from the beginning, is of greater longitudinal than transverse extent (*Carpenter, 1906*). The neurofibrils converge at their point of exit from the midbrain and, in the 30-somite chick, form a loose strand directed ventrally (Fig. 125-B). At the distal end of this strand the fibrils again disperse (*Tello, 1923*). Cell nuclei are intermingled with the fibers along the course of the nerve (*Carpenter, 1906*; *Campenhout, 1937a*). The oculomotor is the largest motor nerve present at this time (*Windle and Austin, 1936*). In embryos of approximately this stage, it reaches no farther than the infundibulum (*Carpenter, 1906*), but its growth is so rapid that it is very soon found to be directed cranially (*Campenhout, 1937a*).

In 35- to 36-somite embryos (of 70 to 72 hours' incubation), or even in younger ones of 59 to 65 hours (*Campenhout, 1937a*), the oculomotor nerve has extended forward and outward as far as the tissues surrounding the posterior surface of the eye vesicle (*Tello, 1923*), and it is provided with cells of Schwann throughout its entire length (*Campenhout, 1937a*). Close to its end, the oculomotor nerve gives off a branch whose fibers mingle with fibers of the ophthalmic branch of the trigeminal nerve (*Tello, 1923*; *Campenhout, 1937a*). At this time there appears the first indication of the ciliary ganglion. According to *Tello (1923)*, it is located at the distal end of the oculomotor nerve. *Campenhout (1937a)* regarded the early ciliary ganglion as situated at the end of the ophthalmic, rather than at the end of the oculomotor nerve, and noted that the ganglion has a nerve connection with the maxillomandibular lobe of the semilunar ganglion in the 72-hour chick embryo. *Carpenter (1906)*, on the other hand, placed the ciliary ganglion on the lateral aspect of the oculomotor nerve and stated that it was a ramus of the ophthalmic nerve, not its main trunk, which joined the ganglion.

By the fifth day, the ciliary ganglion is located about two thirds of the way from the proximal to the distal end of the oculomotor nerve (*Carpenter, 1906*). The ciliary nerve, proceeding from the ganglion to the iris, was observed by *Campenhout (1937a)* in the 96-hour chick, although *Carpenter (1906)* was unable to find it before the eighteenth day.

On the fourth day, a few axons of the oculomotor nerves may cross through the ventral commissure (*Windle and Austin, 1936*). *Bok (1915)* noted that, at the end of the fourth incubation day, the nuclei of the oculomotor nerves are each divided into two cell groups, one dorsal and the other ventral or ventromedial. In the 5-day chick, he observed fibers connecting the ventral nuclei, and he stated that this commissure increased in size until the sixteenth day of incubation. As the floor of the mesencephalon increases in thickness, the oculomotor nuclei are brought to a considerably more dorsal position, and the nerve fibers pass through



a continually increasing quantity of brain substance before making their exit.

On the fifth day, the course of the oculomotor nerve from its point of origin is caudalward, ventralward, and slightly lateralward as far as the optic stalk. The nerve then turns medially and courses ventral to the inferior oblique eye muscle (*Carpenter, 1906*). *Carpenter (1906)* observed that no branches reach the primordia of the other eye muscles at this time. However, *Marshall (1878)* stated that the internal rectus muscles of the 5-day chick are reached by the posterior or main branch of the nerve, which he believed developed first, and that the superior rectus muscle is innervated almost as soon by a smaller anterior branch. According to *Carpenter (1906)*, fibrils can be seen running from the nerve to the superior and inferior rectus muscles on the seventh day, but not to the internal rectus.

### *The Trochlear Nerves*

The trochlear (cranial IV) nerves are special somatic motor nerves. Each innervates the superior oblique muscle of the eye of the contralateral side. The nerves cross in the dorsal surface of the brain at the boundary between the midbrain and the hindbrain. During embryonic development, the nuclei originate within the brain wall a short distance caudal and dorsal to the nuclei of the oculomotor nerves. Like the latter nuclei, those of the trochlear nerves appear to move dorsalward as development progresses.

Differentiation of neuroblasts in the nucleus of the trochlear nerve of the chick begins some time prior to 72 hours of incubation (*Tello, 1923*), probably at about 60 hours, or the 32-somite stage (*Bok, 1915; Windle and Austin, 1936*). In the 36-somite chick each nucleus consists of a group of bipolar neuroblasts (*Fabre and Mégevand, 1941*) situated close to the midline and dorsal to the median longitudinal fasciculus. Fibers proceed outward from the nucleus at right angles to the longitudinal axis of the brain stem and then turn dorsally, following the course of the forming mesorhombencephalic sulcus (*Tello, 1923, 1924a*). At some time between the seventy-ninth and eighty-fifth hour (*Bok, 1915; Tello, 1924a; Campenhout, 1937a*), the nerves, having crossed the dorsal midline, leave the brain and start to grow ventrally over the lateral face of the mesencephalon. At the end of the fourth day, according to *Fabre and Mégevand (1941)*, there is some mingling of fibers of the crossed tectobulbar tract with those of the as yet uncrossed trochlear nerve fibers. *Windle and Austin (1936)* also noted that trochlear nerve fibers appear to be joined by other fibers originating in the mesencephalic root of the trigeminal nerve.

The trochlear nerves, in growing toward their end organs, seem to



follow the course of the more superficial blood vessels (*Tello, 1924a; Fabre and Mégevand, 1941*).

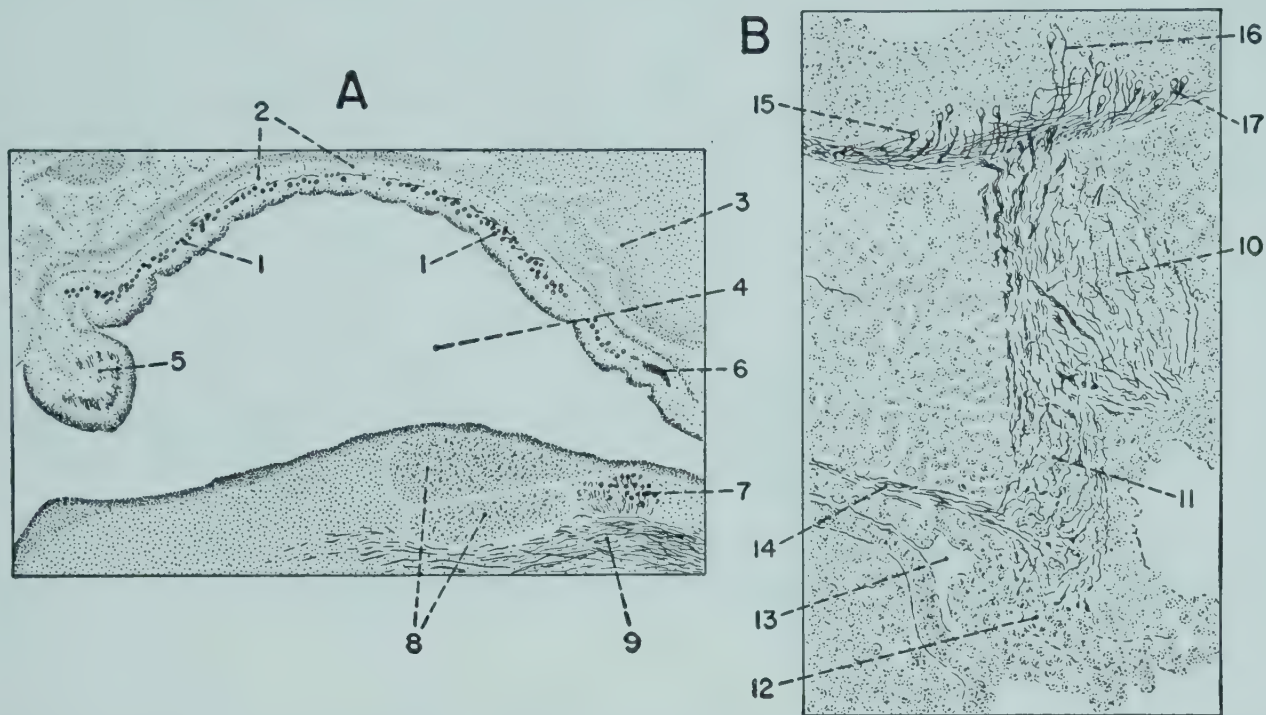
### *The Trigeminal Nerves*

The trigeminal (cranial V) nerves are rather small in birds. They are the most anterior pair of mixed cranial nerves. Each nerve contains general somatic sensory and special visceral motor elements. As the trigeminal nerve emerges from the brain stem it enters a large, bilobate ganglion, the semilunar or Gasserian ganglion. Distal to the ganglion the nerve is divided into three branches: the ophthalmic nerve, the maxillary nerve, and the mandibular nerve. The first two of these nerves are purely sensory; the mandibular nerve, however, receives an accession of fibers from the motor division of the trigeminal nerve. The trigeminal nerve is sensory to the skin of the face and bill, the mucous membranes of the mouth and nose, the orbital structures, and the dura. The motor division innervates the adductor muscles of the bill. General visceral sensory (proprioceptive) elements which supply the muscles of mastication arise from cells in the mesencephalon and contribute to the trigeminal nerve.

As previously remarked, the neural crest is of exceptionally large size at the trigeminal level of the 13-somite chick embryo. A few hours later, when the embryo possesses 17 to 18 somites (*Campenhout, 1937b, 1937c*), cells begin to bud off the inner surface of the epithelial ectoderm and to penetrate into the mesenchyme dorsolateral to the rhombencephalon, at a point about one third of the way down from the dorsal midline of the head. As yet, these epithelioid cells make no contact with the mesenchymatoid cells in the cell column, or "periaxial cord," of neural crest origin. As development proceeds, the budding process gives rise to a continually larger number of cells, and at some time between the 21- and 25-somite stage an intermingling of these cells with those of the cellular column takes place (*Campenhout, 1937b, 1937c*). *Goronowitsch (1893)* signified, however, that this intermingling may occur as early as the 13-somite stage. Simultaneously, the area of budding migrates ventrally and takes up an epibranchial position. As the ectodermal budding continues to contribute cells to the distal portion of the cellular column, the character of the latter becomes predominantly epithelioid, while the more proximal portion remains largely mesenchymatous. At the 29- to 30-somite stage, the distal and proximal portions of the cellular column become recognizable as the anlagen of the ophthalmic and maxillomandibular lobes, respectively, of the semilunar ganglion. The distal portion enlarges in the cranial direction and the proximal portion in the caudal direction. The maxillomandibular lobe, however, does not retain its mesenchymatous character, since some of the mesenchymatoid cells de-



generate, and the contribution of epithelial cells is augmented (*Campenhout, 1937a, 1937c*). At 60 hours (probably about the 33-somite stage), the two lobes are of identical composition (*Campenhout, 1937c*). At this time, the ophthalmic lobe becomes entirely independent of the ectoderm, as does the maxillomandibular lobe at the seventy-eighth hour. The two lobes, fused at their base, are then clearly evident (*Kastschenko, 1887; Campenhout, 1937c*).



**Fig. 127.** Early stages in the development of the mesencephalic root of the trigeminal nerve and the acoustico-facial ganglion. (Redrawn with modifications after Tello, 1923.)

A, a median sagittal section through the brain of the 11-day chick embryo, taken in the region of the aqueduct of Sylvius and including the mesencephalic root of the trigeminal nerve ( $\times 30$ ); B, a section through the acoustico-facial ganglion of the 72-hour chick embryo ( $\times 150$ ).

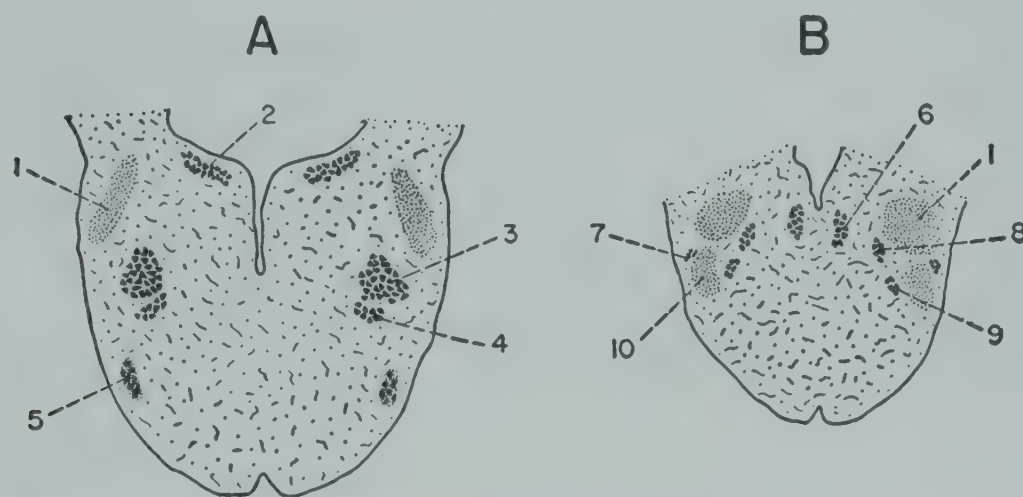
1, mesencephalic trigeminal root; 2, tectal commissure; 3, cerebellum; 4, aqueduct; 5, posterior commissure; 6, decussation of trochlear nerve; 7, nucleus of trochlear nerve; 8, oculomotor nerve; 9, medial longitudinal fasciculus; 10, ganglionic portion of acoustic nerve; 11, ganglionic portion of facial nerve (sensory division or intermediary nerve); 12, epibranchial placode; 13, first branchial pouch; 14, initial fibers of chorda tympani; 15, homolateral neuroblasts of sensory nucleus; 16, fiber of future tractus solitarius; 17, arcuate neuroblasts of alar region.

In the meantime, at the 20- to 25-somite stage, the cells of both portions of the semilunar ganglion, especially the ophthalmic portion, have begun to undergo differentiation into bipolar neuroblasts. A few proximal fibrils originating in the ophthalmic lobe penetrate into the first neuromere of the rhombencephalon, thus initiating the development of the descending tract of the trigeminal nerve. The ophthalmic nerve is represented by a strand of cells, showing fine neurofibrillar differentiation, which extends from the posterior limit of the mesencephalon as far craniad as the optic vesicle (*Tello, 1923*). At the same time, the first fibers of the motor



division of the trigeminal leave the ventral part of the rhombencephalon (*Windle and Austin, 1936*). At the 30-somite stage, these enter the mandibular portion of the semilunar ganglion. The peripheral processes of the bipolar neuroblasts in this lobe have, by this time, entered the corresponding branchial arch (the mandibular process of the first arch), and the proximal processes are in contact with the neural tube (*Tello, 1923*). In the 37-somite chick, some of the sensory fibers of the trigeminal nerve ascend a short distance in the medulla and others descend (*Windle and Austin, 1936*).

At the beginning of the fourth day (when there are about 35 somites), the mandibular branch of the trigeminal nerve starts to divide into the



**Fig. 128.** Diagrammatic representations of cross sections through the medulla of the 12-day chick embryo taken at two different levels. (Redrawn after Eckardt and Elliott, 1935.)

A, the motor nuclei of the trigeminal nerve; B, the motor nuclei of the abducens and facial nerves.

1, descending root of trigeminal nerve; 2, dorsal motor nucleus of trigeminal nerve; 3, dorsal division of ventral motor nucleus of trigeminal nerve; 4, ventral division of same; 5, lateral lemniscus; 6, principal motor nucleus of abducens nerve; 7, accessory motor nucleus of abducens nerve; 8, dorsomedial motor nucleus of facial nerve; 9, ventrolateral motor nucleus of facial nerve; 10, superior olivary muscles.

maxillary and mandibular nerves (*Tello, 1923*). At the end of the fourth day the ophthalmic branch may be traced forward to a point close to the olfactory nerve (*Marshall, 1878*). It lies lateral to the oculomotor nerve, which it crosses at right angles, and dorsal to the optic nerve and all of the eye muscles except the superior rectus, under which it passes (*Marshall, 1878*). The posterior or mandibular branch can be followed along the anterior border of the mandibular arch. The maxillary branch is given off close to the distal extremity of this branch and passes into the maxillary process of the first pharyngeal arch.

The mesencephalic trigeminal root, according to *Windle and Austin (1936)*, can be seen in the chick on the fourth day in the posterolateral wall of each optic lobe. The cells of this nucleus give rise to axons which mingle with fibers of the trochlear nerve. On the fifth day its fibers reach



the motor nucleus of the trigeminal nerve. The growth of these fibers thus appears to be much more rapid than reported by Tello (1923), who found that they had reached no farther than the decussation of the trochlear nerve by the seventh day. At this time (7 days) the cells from which the mesencephalic trigeminal root arises can be seen on either side of the midline in the postcommissural recess and in the septum dividing the optic lobes. On the eleventh day (Fig. 127-A), after the formation of the aqueduct of Sylvius, the cells are spread out longitudinally along the roof of the aqueduct (Tello, 1923).

There are differing reports regarding the order of appearance of the various motor nuclei of the trigeminal nerve of the chick. Tello (1923) distinguished a dorsal and ventral motor nucleus on the fourth day as did Bok (1915) on the fifth, whereas Eckardt and Elliott (1935) stated that the dorsal nucleus appears on the sixth day. The latter authors observed that the ventral nucleus, undivided on the fifth day, has separated into dorsal and ventral portions after the eleventh day (Fig. 128-A). In the 4-day chick, Windle and Austin (1936) found a lateral motor V nucleus, in addition to the medial nucleus, at the point of emergence of the motor root.

### *The Abducens Nerves*

The abducens (cranial VI) nerves are special somatic motor nerves and innervate the lateral rectus muscles, the retractor bulbae muscles, and the nictitating membranes.

The initial development of the abducens nerve was observed by Windle and Austin (1936) in the 32-somite chick at the sixtieth hour of incubation, although other investigators have placed its origin anywhere from the seventy-second to the ninety-third hour (Marshall, 1878; Carpenter, 1906; Bok, 1915; Tello, 1923). It arises near the midline from the ventral side of the rhombencephalon (Carpenter, 1906). The nerve is slender and has a number of attenuated roots ranged longitudinally one behind the other. It passes out of the brain in the ventral direction, turns anteriorly, and courses forward parallel to the ventral surface of the rhombencephalon. Carpenter (1906) found that it has attained the primordium of the lateral rectus muscle in chicks of 88 to 93 hours of incubation. He also stated that indifferent medullary cells migrated out into the root of the abducens nerve to give rise to cells of Schwann.

The abducens nerve has two nuclei: a chief nucleus, which, in the 5-day chick, is found ventral to the floor of the fourth ventricle and lateral to the median raphe; and an accessory nucleus, which appears by the seventh day between the descending root of the trigeminal nerve and the superior olivary nucleus (Fig. 128-B)—that is, far lateral to the chief nucleus and close to the surface of the brain (Eckardt and Elliott, 1935). The accessory



nucleus apparently is formed by the migration of cells from the chief nucleus (*Levi-Montalcini, 1942*).

### *The Facial Nerves*

The facial (cranial VII) nerves are mixed visceral sensory and visceral motor nerves. The major root of the nerve is special visceral motor and arises from two motor nuclei in the medulla oblongata: a dorsal nucleus just caudoventral to the masticator nucleus of the trigeminal nerve, and a ventral nucleus just lateral to the superior olive. Scattered cells often are seen connecting these two nuclear groups. The fibers of the major root innervate the superficial musculature of the face and scalp, the depressor muscles of the bill and the constrictor colli muscles. The minor root of the facial nerve forms the glossopalatine or intermediate nerve of Wrisberg, most of whose fibers enter the chorda tympani nerve. Some special visceral sensory fibers from the taste buds of the anterior one third of the tongue reach the brain via this pathway. General visceral motor fibers (preganglionic autonomic) to the submaxillary and submandibular glands pass in this root. The sensory fibers of this nerve originate in the geniculate ganglion.

The facial portion of the neural crest, recognizable in the chick at the 13-somite stage, grows in the ventral direction and, in the 17-somite chick, has reached the upper end of the hyoid arch. It is composed of mesenchymatous tissue which is much denser than the mesenchyme itself and is therefore easily distinguishable (*Campenhout, 1937a*). In the 26-somite chick, a very small number of bipolar neuroblasts have differentiated in this mass of tissue, and the axons of two or three have penetrated into the third rhombomere (*Tello, 1923*). The cellular column derived from the facial part of the neural crest now extends throughout the hyoid arch anterior to the otic vesicle. Cells are beginning to bud from the inferior surface of the ectoderm overlying the cell column, but the area where budding first occurs is not as far dorsal as the region in which it is initiated over the trigeminal division of the neural crest. By the 30-somite stage the region of budding is epibranchial in position, and contact has been established between the ventral extremity of the cell column and the cells of ectodermal origin (*Campenhout, 1937a*). At the same time, or perhaps earlier, at the 27-somite stage (*Windle and Austin, 1936*), the first motor fibers of the facial nerve penetrate into the primordial ganglion from the motor nucleus in the third rhombomere (*Tello, 1923*).

Prior to the 30-somite stage, there is no contact between the facial cell column and the auditory epithelium. Between the 30- and the 35-somite stages, the dorsocaudal surface of the proximal part of the column becomes clearly applied to the inner surface of the otic vesicle. At this time, cells are leaving the auditory epithelium to form the acoustic



ganglion, and fusion of the facial and acoustic cell columns now takes place (*Campenhout, 1937a*). This fusion is thus a secondary occurrence, the two portions of the neural crest being separate in the beginning (*Goronowitsch, 1893*), contrary to earlier beliefs (*Marshall, 1878; Béraneck, 1887*).

During this period, the budding of cells from the placode over the hyoid arch becomes very pronounced. The distal portion of the cell column fuses with these cells and forms a ganglionic swelling close to, but distinct from, the acoustic ganglion (*Campenhout, 1937a*). This is the anlage of the geniculate ganglion. The derivation of the geniculate ganglion from the ectodermal placode over the hyoid arch rather than from neural crest cells is indicated by the results of experiments. Extirpation of the placode at the 18-somite stage prevents the formation of the ganglion, but removal of the neural crest between the 4- and 14-somite stages has no effect upon its development. The trunk of the facial nerve, however, does not appear in the absence of the neural crest (*Yntema, 1944*).

In the 35- to 36-somite (72-hour) chick the primitive acoustico-facial ganglionic complex has roughly the shape of an inverted Y (Fig. 127-B). The peripheral processes of the cells in the geniculate ganglion terminate in the ectodermal placode. The proximal processes enter the third rhombomere, where they either turn caudally or continue medially, in the latter case to initiate the differentiation of the tractus solitarius. A few fibers leave from a point close to the distal end of the ganglion, forming the early chorda tympani nerve (*Tello, 1923*), a small branch to the tongue.

The two components of the acoustico-facial ganglionic complex now begin to separate clearly. In the 96-hour chick, the geniculate ganglion is attached to the rhombencephalon by a long nerve root and is still intimately applied to the ectoderm, into which it appears to send numerous nerve fibrils (*Campenhout, 1937a*).

The motor nucleus of the facial nerve is a single cell group in the 5-day chick but has separated into a dorsomedial and ventrolateral group (cf. Fig. 128-B) by the seventh incubation day (*Eckardt and Elliott, 1935*). The fibers leave the nucleus in a dorsal direction. Most of them turn laterally and emerge on the homolateral side, but a few turn medially and cross to the opposite side of the brain before emerging (*Bok, 1915*).

The descending sensory fibers of the facial nerve lie originally in the marginal layer between the descending trigeminal and vestibular tracts. The facial elements then join those of the trigeminal tract (*Bok, 1915*).

### *The Acoustic Nerves*

The acoustic (cranial VIII) nerves consist of special somatic sensory components only. In the adult, each nerve has a cochlear branch subserv-



ing audition and a vestibular branch subserving proprioceptive functions. The cochlear nerve arises from the spiral ganglion and the vestibular nerve from the ganglion of Scarpa (or vestibular ganglion). These ganglia and both nerves originate during embryogenesis from the primitive acoustic ganglion.

As already stated, the connection between the facial and acoustic ganglionic anlagen is apparently established secondarily in the chick at about 57 hours of incubation (approximately the 30- to 31-somite stage), when the dorsal and caudal portion of the facial cell column or periaxial cord becomes applied to the medial surface of the otic vesicle (*Campenhout, 1937a*). The primitive acoustic ganglion is then formed of cells budded off from the auditory epithelium; the neural crest apparently does not participate in the development of this ganglion (*Campenhout, 1939*). The first indication of the budding process is detectable at the 21-somite stage, when a few cells in the basal layer of the thickened epithelium at the bottom of the open auditory pit become partially detached and, at the same time, rounder. By the sixtieth hour of incubation, the budded cells begin to migrate into the underlying mesenchyme (*Campenhout, 1937a*). The inverted Y formation of acoustico-facial ganglion complex of the 70- to 72-hour chick (cf. Fig. 127-B) has already been described. In the chick of this age, the acoustic portion of the complex contains small cells medially and large ones distally, and it sends nerve fibrils into the epithelium of the otic vesicle (*Campenhout, 1937a*). The large cells represent the vestibular portion of the ganglion and the small cells the spiral (cochlear) portion (*Baumann, 1947*).

Early in the fourth day of incubation, the acoustic ganglion consists of two portions and has become entirely independent of the facial nerve, although the roots of the acoustic and facial nerves still form a common trunk. At the end of the fourth day, the ganglion is large, bilobate, and closely applied to the auditory epithelium (*Campenhout, 1937a*). The lagena of the ear has started to develop, and the portion of the ganglion in contact with it will differentiate as the spiral ganglion (*Bok, 1915*). By the seventh incubation day, this ganglion and the ganglion of Scarpa are separate and distinct (Fig. 129-A).

**The Vestibular Nerves.** The vestibular nerves develop before the cochlear nerves. According to Windle and Austin (1936), the ascending and descending vestibular tracts are initiated in the (32-somite) chick embryo at 60 hours of incubation, when central processes of cells in the acoustic ganglion reach the neural tube. At 72 hours, fibers seeming to originate from the vestibular part of the ganglion pass medially as well. At the end of the fourth incubation day, the ascending vestibular tract almost reaches the cerebellar anlage. A few of its fibers attain the cerebellum 24 hours later (*Windle and Austin, 1936*). The descending tract,



which lies closer to the midline of the medulla than the ascending tract (Bok, 1915), can be followed nearly to the level of the vagus nerve in the 5-day embryo (Windle and Austin, 1936). A secondary descending path-

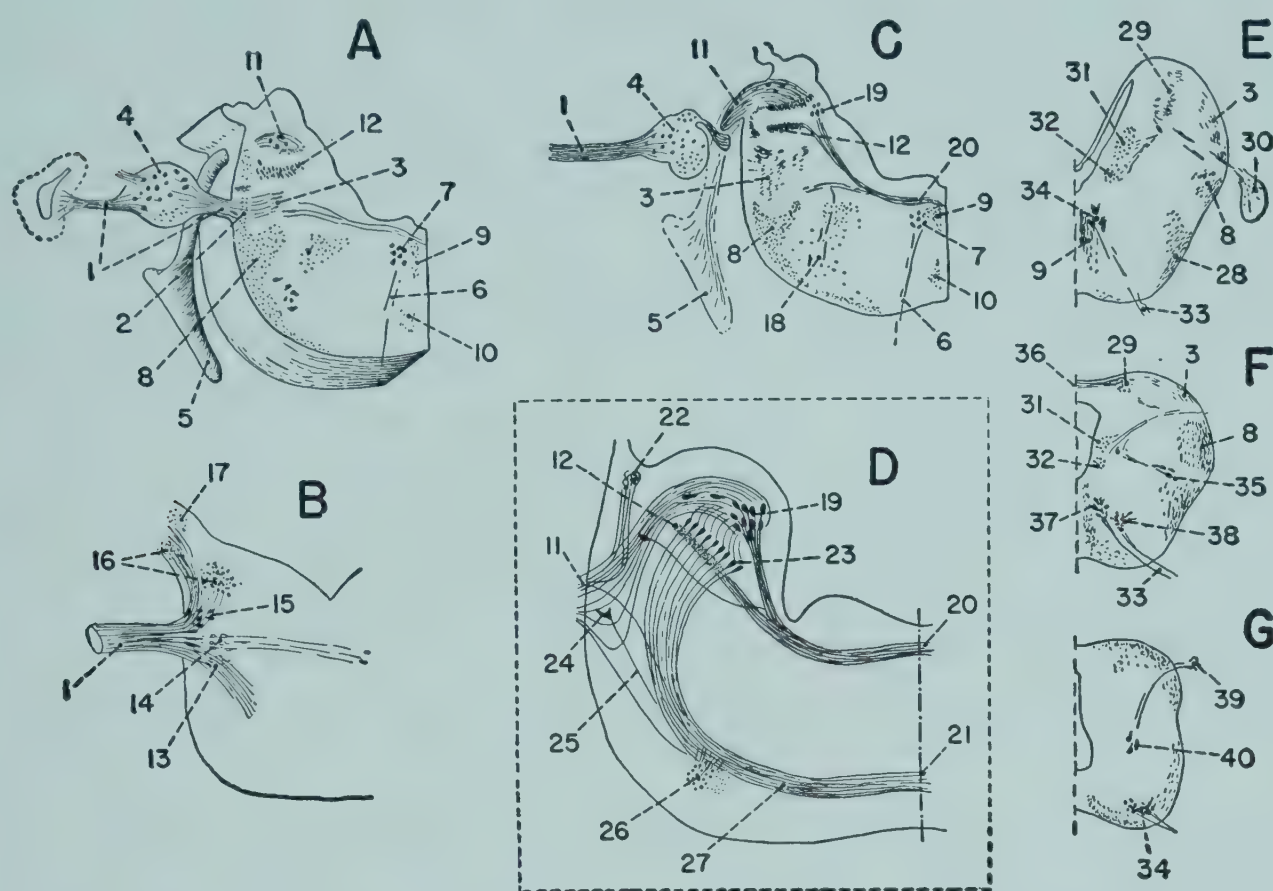


Fig. 129. The developing nuclei of various cranial nerves in the chick embryo, as seen in cross sections of the medulla. (Redrawn with modifications A, C, D, E, F, and G, after Bok, 1915; B, after Levi-Montalcini, 1949).

A, the vestibular centers of the 7-day embryo ( $\times 15$ ); B, the vestibular centers of the 11-day embryo ( $\times 6$ ); C, the cochlear centers of the 7-day embryo ( $\times 15$ ); D, the cochlear centers of the hatched chick; E, F, G, sections through the nuclei of the vagus, spinal accessory, and hypoglossal nerves of the 7-day embryo ( $\times 15$ ).

1, vestibular nerve; 2, ascending root of vestibular nerve; 3, descending root of vestibular nerve; 4, ganglion of Scarpa; 5, spiral ganglion; 6, fibers of abducens nerve; 7, nucleus of abducens nerve; 8, descending root of trigeminal nerve; 9, medial longitudinal fasciculus; 10, crossed tectobulbar tract; 11, cochlear nerve or fibers; 12, cochleo-cerebellar tract; 13, nucleus vestibulo-descendens; 14, tangential nucleus; 15, lateral vestibular nucleus (of Deiters); 16, dorsolateral vestibular nucleus; 17, nucleus vestibulo-cerebellaris; 18, nucleus of facial nerve; 19, nucleus magnocellularis; 20, dorsal cochlear commissure; 21, ventral cochlear commissure; 22, nucleus angularis; 23, nucleus laminaris; 24, ventral cochlear nucleus; 25, primary cochlear fibers in ventral system; 26, superior olive and lateral lemniscus; 27, trapezoid body; 28, homolateral tectobulbar tract; 29, tractus solitarius; 30, vagus nerve; 31, dorsal motor nucleus of vagus nerve; 32, nucleus intermedius; 33, hypoglossal nerve; 34, nucleus of hypoglossal nerve; 35, ventral motor nucleus of vagus nerve; 36, commissura infima; 37, dorsal nucleus of hypoglossal nerve; 38, ventral nucleus of hypoglossal nerve; 39, spinal accessory nerve; 40, nucleus of spinal accessory nerve.

way, the vestibulo-spinal tract, originating from the lateral vestibular nucleus (of Deiters) in the medulla, is plainly visible on the sixth day (Visintini and Levi-Montalcini, 1939).



On the eighth day (*Visintini and Levi-Montalcini, 1939; Levi-Montalcini, 1949*), fibers of large diameter—the “colossal” fibers of Ramon (*1908a*)—grow into the medulla from the vestibular ganglion. After the eleventh day, some of them form synaptic connections with cells of the tangential or intercalary nucleus, which lies immediately inside the medulla, and which is the point of origin of a crossed descending vestibular tract, visible after the eleventh day (*Visintini and Levi-Montalcini, 1939*). These synaptic connections are unusual in that the neurones of the tangential nucleus apply themselves lengthwise against the colossal fibers (*Visintini and Levi-Montalcini, 1939*); then, according to the account given by Ramon (*1908c, 1908d*), the colossal fibers emit short, thick branches which terminate in concave disks applied to the bodies of the tangential cells. It is of interest to note, however, that the existence of the colossal fibers has been denied on the grounds that they are artifacts (*Baumann, 1947*). Also, the peculiar appearance of the synapses has been attributed to misinterpretation of the special structure of the cells forming the tangential nucleus (*Baumann, 1947; Niederhäusern, 1947*). This misinterpretation is said to be due to the formation of a fissure which, from the eighth day onward, gradually separates the body of each bipolar tangential cell from its median process; thus, by the twelfth day, the fissure has reached the point of attachment of the peripheral process, and the peripheral and median processes have united with each other and are connected with the cell body only by a very short pedicle (*Baumann, 1947*).

As to the development of the peripheral portion of the vestibular nerve in the chick, Tello (*1923*) has stated that the peripheral processes of the bipolar cells in the vestibular ganglion can be seen at 72 hours converging externally. Some then penetrate into the wall of the otic vesicle, probably as the first fibers of the superior or anterior branch of the vestibular nerve. Others, representing the early posterior branch of the nerve, extend around the wall of the otocyst to the primordium of the posterior semicircular canal.

There are slightly differing accounts of the development of the peripheral fibers of the vestibular nerve after the fourth day. According to Visintini and Levi-Montalcini (*1939*), plexuses of nerve fibers can be found in the 5-day embryo applied to the base of the sensory epithelium in the ampullae of the semicircular canals. On the sixth and seventh days the fibers terminate between the epithelial cells, and on the eighth day colossal fibers reach the cristae from the vestibular ganglion. Baumann (*1947*), however, found that individual nerve fibers had penetrated among the stratified epithelium of the cristae at 4 days, and that some had formed terminal bifurcations against the base of the most internal cellular layer. At 7 days a plexus existed. This author stated that all fibers in the cristae were of the same caliber on the ninth day.



The vestibular centers in the medulla are as follows: the nucleus vestibularis tangentialis, situated laterally and consisting of neurones arranged in rows between the entering vestibular fibers; the nucleus vestibularis ventrolateralis or nucleus of Deiters, medial to the tangential nucleus; the nucleus vestibularis descendens, within the descending root of the nerve; (probably) the nucleus vestibularis dorsomedialis, lying medial to the nucleus magnocellularis of the cochlear nerve; the nucleus vestibularis dorsolateralis, more or less on the course of the ascending root of the nerve; and the nucleus vestibularis superior, in the most dorsal position. The large dorsolateral nucleus is subdivided into several nuclear masses. The root fibers appear to have direct connection with all the above nuclei (*Levi-Montalcini, 1949*).

The time of appearance of the vestibular nuclei in the chick embryo cannot be stated exactly, but most of them are present by the eleventh day (Fig. 129-B). The lateral vestibular nucleus (of Deiters) presumably starts to develop between the sixth day (*Visintini and Levi-Montalcini, 1939*) and the eighth day (*Baumann, 1947*), and the tangential nucleus on approximately the seventh day (*Baumann, 1947; Levi-Montalcini, 1949*).

The origin of the tangential nucleus is obscure. It has been said to form from cells that migrate out of the acoustic ganglion into the medulla (*Neiger, 1950*), as well as from medullary cells which migrate from a medial to a lateral position—that is, in exactly the reverse direction (*Levi-Montalcini, 1949*). After early extirpation of the otic vesicle, the tangential nucleus is the only vestibular center which fails to appear, although certain cells of very doubtful identity perhaps represent a vestige of it (*Levi-Montalcini, 1949*). This observation tends, more or less, to support the former of the two theories mentioned above.

**The Cochlear Nerves.** According to *Windle and Austin (1936)* the cochlear apparatus is absent in the 5-day chick embryo, but *Bok (1915)* has stated that cochlear fibers can be found at the end of the fourth day of incubation arising from the neuroblasts of the spiral ganglion. These fibers course over the laterodorsal surface of the (future) ganglion of Scarpa without making any contact with the vestibular fibers. A few of them reach and enter the rhombencephalon, and, after turning dorsalward, end in the mantle layer close to the ventricular cavity.

Within the rhombencephalon the early dorsal cochlear commissure can be identified at the end of the fourth day (*Bok, 1915*). Fibers arise from a group of neuroblasts (later to be clearly recognizable as the nucleus magnocellularis) lying near the limiting sulcus, cross the midline dorsal to the median longitudinal fasciculus, and end among a group of small cells in the mantle layer ventral to the nucleus magnocellularis of the opposite side. This latter group of small cells will become the nucleus



laminaris, which cannot be recognized until the seventh day (*Williams, 1937*).

In the 7-day embryo, the nucleus magnocellularis is well defined (Fig. 129-C). Some of the fibers originating from its cells turn cranialward after reaching the vicinity of the nucleus laminaris of the opposite side. According to Bok (1915), these fibers constitute the early cochleo-cerebellar tract. Later, synaptic connections form between the nucleus laminaris and the homolateral nucleus magnocellularis (*Bok, 1915; Levi-Montalcini, 1949*).

On the eighth day the cochlear nerve branches immediately after entering the brain. Short, dorsally directed fibers terminate at the nucleus angularis, which has appeared in the dorsolateral angle of the medulla. Most of the remaining fibers now reach the nucleus magnocellularis. Unlike Ramon (1908d), Bok (1915) found that a few fibers of the cochlear root turn ventralward, then medialward, and proceed to the superior olivary nucleus. Bok (1915) also stated that the cochleo-cerebellar tract has attained the nucleus lateralis in the cerebellum at this time, but added that it is less readily distinguishable from the tenth day onward.

On the ninth day ganglionic cells forming the ventral cochlear nucleus are found among the fibers of the cochlear nerve immediately after its entrance into the brain. Fibers from these ganglionic cells proceed ventromedially to the superior olivary nucleus. A few cells which apparently have migrated laterally from the nucleus magnocellularis are now intermingled with the entering fibers of the cochlear nerve. Fibers from these cells pass on the ventral side of the nucleus laminaris to join the dorsal cochlear system (*Bok, 1915*).

During some later period, but apparently before the eleventh day (*Levi-Montalcini, 1949*), the nucleus laminaris gives rise to ventromedially directed fibers. These fibers (which constitute the trapezoid body) give off collaterals to the homolateral superior olivary nucleus, and then cross the midline to ascend in the lateral lemniscus of the opposite side. The ventral cochlear commissure thus appears.

The nucleus angularis, at least up to the eleventh day, appears to make connections with no fibers other than those of the cochlear root. Eventually, however, fibers from this nucleus seem to proceed to the homolateral and contralateral nucleus laminaris. Between the eleventh and twenty-first days, this nucleus undergoes a tenfold increase in size, due to growth of its cells rather than to the addition of new elements (*Levi-Montalcini, 1949*).

When the root fibers of the cochlear nerve are removed, the cochlear centers of the medulla develop normally until the eleventh day. Thereafter, the number of cells in the nucleus angularis decreases by more than 80



per cent, and the nucleus magnocellularis suffers the loss of 30 per cent of its elements, apparently because of the lack of functional stimuli. The nucleus laminaris, however, is unaffected, at least up to the seventeenth day (*Levi-Montalcini, 1949*). These findings tend to confirm the suspected absence of direct connections between the cochlear fibers and the nucleus laminaris.

The complexities of the cochlear system are in need of further investigation. Bok's (1915) diagram of this system is shown in Fig. 129-D.

### *The Glossopharyngeal and Vagus Nerves*

The glossopharyngeal (cranial IX) and vagus (cranial X) nerves are so closely related in development that they will be discussed together. Both are general and special visceral motor and sensory nerves with some general somatic sensory elements. The glossopharyngeal nerve innervates pharyngeal muscles and the salivary glands, and the vagus nerve supplies the cervical, the thoracic, and a large part of the abdominal viscera.

A large ganglionic complex is present on the roots of these two nerves. The more rostral portion of this complex is the superior ganglion of the glossopharyngeal nerve, and the more caudal portion is the jugular ganglion of the vagus nerve. In addition, a ganglion is present on the trunk of each nerve a short distance from the brain. The trunk ganglion of the glossopharyngeal nerve is the petrosal ganglion, and that of the vagus nerve is the nodose ganglion.

It is generally accepted that the most rostral part of the postotic neural crest is a source of material entering into the development of cranial nerves IX and X (*Marshall, 1878; Campenhout, 1937a, 1937b*); Chiarugi (1890) recognized a distinct vagus component. Whether or not neural crest cells provide any elements other than cells of Schwann cannot be definitely stated (*Campenhout, 1937a*). Tello (1946) expressed the opinion that no cells of neural crest origin become neuroblasts of the vagus nerve. The visceral sensory components of the two nerves appear to be derived principally from cells of the petrosal and nodose ganglia, respectively. These ganglia are formed exclusively of cells contributed by epibranchial placodes (*Campenhout, 1937a, 1937b; Levi-Montalcini, 1946b*). Experimental evidence indicates that the root ganglia, on the other hand, take their origin from the neural crest (*Levi-Montalcini, 1946b*).

In the chick of 17 to 18 somites, the cells of the glossopharyngeal-vagal portion of the neural crest have already undergone considerable latero-ventral migration beneath the ectoderm and over the external surface of the first and second somites. By the time the 26- to 27-somite stage is reached, the distal portion of the migratory cell column has started to



bifurcate, the anterior branch being directed toward the third visceral arch and the posterior toward the fourth (*Campenhout, 1937a*). In the 55-hour (probably 30-somite) chick, the distal extremities of the two cell columns are fused with the epibranchial ectoderm (*Kastschenko, 1887; Campenhout, 1937a*). At 59 hours of incubation, cells from the epibranchial ectoderm have begun a massive migration into the glossopharyngeal column, and soon afterward a few cells bud off the inner surface of the epiblastic placode of the fourth visceral arch (*Campenhout, 1937a; Tello, 1946*). Many of the mesenchymatoid cells of neural crest origin now seem to be degenerating. At 65 hours (or approximately the 34-somite stage), it is apparent that those cells which are not degenerating are being transformed into cells of Schwann, but it has not been determined whether or not they are also the source of the cells now undergoing neurofibrillar differentiation in the glossopharyngeal cell column (*Campenhout, 1937a, 1937b; Tello, 1946*). By the third day of incubation, the root ganglia have developed (*Levi-Montalcini, 1946b*), the jugular ganglion first appearing as a row of small swellings decreasing progressively in size from anterior to posterior (*Kastschenko, 1887*).

The first sensory neurofibrils have been observed in the ganglionic anlagen of nerves IX and X in chick embryos with as few as 22 (*Tello, 1946*) and 32 somites (*Windle and Austin, 1936*). In the 72-hour (36-somite) chick, the proximal processes of the ganglionic neuroblasts enter the brain, forming several separate roots. According to one account (*Windle and Austin, 1936*), the fibers, at this stage, end almost immediately after passing into the rhombencephalon. *Tello (1923)*, however, found that fibers originating from cells in the petrosal ganglion penetrate into the fifth and sixth rhombomeres and initiate a descending pathway, although only a few fibrils arising from the nodose ganglion as yet enter the brain. After 4 days of development, sensory fibers of the glossopharyngeal and vagus nerves contribute largely to the tractus solitarius (*Tello, 1923*), which, at 5 days of incubation, can be followed caudally as far as the spinal cord (*Windle and Austin, 1936*). Fibers of the glossopharyngeal nerve and the most rostral roots of the vagus cross the midline of the brain dorsal to the medial longitudinal fasciculus and end in the heterolateral tractus solitarius. After the fifth day, the most posterior fibers of the vagus form the commissura infima which crosses in the roof of the medulla (*Bok, 1915*). In the 9-day embryo, the tractus solitarius consists of a dorsal and a ventral bundle, which give off fibers to form two sensory nuclei within the medulla (*Bok, 1915*).

Neuroblasts of the vagus and glossopharyngeal motor nuclei have been identified in the rhombencephalon at the 22- and 27-somite stages, respectively (*Windle and Austin, 1936*). The first very small motor rootlets leave the brain at the 32-somite stage (*Windle and Austin, 1936*). In



the 36-somite (72-hour) embryo the motor nuclei extend through the caudal portion of the fifth rhombomere and all of the sixth (*Tello, 1923*).

The later history of these motor nuclei, as well as that of the motor nuclei of the hypoglossal nerve, is not entirely clear. According to the account given by Bok (*1915*), the nuclei of nerves IX and X, after the fifth day, are continuous with each other anteroposteriorly and occupy a dorso-medial position very near the ependymal layer. The common nucleus is of considerable longitudinal extent and thus may be considered a column of cells (cf. Fig. 124-A). Fibers originating in it leave the brain almost immediately ventral to the entering fibers. After 7 days of incubation the column of cells constituting the nucleus has divided into a dorsal and a ventral portion. The dorsal portion, lying close to the ependyma, is the motor nucleus of the glossopharyngeal nerve in its anterior portion and continues posteriorly as the dorsal motor nucleus of the vagus nerve. Ventral to the latter nucleus, and also close to the ependyma, is the nucleus intermedius (cf. Fig. 124-B). (After the tenth day, cells of one of the nuclei of the hypoglossal nerve join the nucleus intermedius, which thus becomes a motor nucleus for this nerve as well as for the vagus.) Lateral to the nucleus intermedius and close to the periphery of the medulla is the ventral nucleus of the vagus nerve (cf. Fig. 124-B). The ventral vagus nucleus is of greater caudal extent than the others, and in its posterior portion probably is continuous with the nucleus of the spinal accessory (XI) nerve. Three cross sections through the 7-day chick embryo's medulla are presented in Fig. 129-E, F, and G. These illustrations indicate the relationships between the nuclei of the tenth, eleventh, and twelfth cranial nerves, as observed by Bok (*1915*).

Slightly different findings have been reported by Eckardt and Elliott (*1935*). These observers noted that the motor nucleus of the vagus nerve begins to divide into a dorsal and a ventral portion on the seventh incubation day. The process of division begins midway between the cranial and caudal limits of the nucleus and progresses rostrally until, on the twelfth day, it is complete throughout the rostral half of the nucleus. The newly engendered dorsal and rostral portion of the nucleus is the motor nucleus of the glossopharyngeal nerve; all the remainder of the nucleus is the motor nucleus of the vagus. No ventral motor nuclei of either the ninth or tenth nerves were found. The nucleus intermedius was located ventrolateral to the vagus nucleus and dorsal to the dorsal motor nucleus of the hypoglossal nerve.

Levi-Montalcini (*1950*) observed that the 3-day vagus nucleus is a medioventral column of cells. On the fourth day, its position is more dorsal. Cells destined to form the dorsal motor nucleus now start to migrate dorsally and continue to do so through the seventh day. Some of the re-



maintaining cells migrate ventrolaterally during the same period to establish the nucleus intermedius, and other cells of the original group apparently fail to move from the mediodorsal position in which they are found on the fourth day.

### *The Spinal Accessory Nerves*

The spinal accessory (cranial XI) nerves are special visceral and general somatic motor nerves supplying pharyngeal and shoulder muscles. Their fibers begin on either side of the brain immediately rostral to the dorsal roots of the first typical spinal nerves (*Windle and Orr, 1934*), and course craniad to join those of the vagus nerve.

The most complete description of the spinal accessory nerve in its early stages of development has been given by Chiarugi (1890). According to his account, which agrees with that of Windle and Orr (1934), the primordial eleventh nerve can be found in the 4- to 5-day chick embryo as a strand of intermingled fibers and cells running longitudinally from the ganglion or dorsal root of the third cervical nerve forward to the root of the vagus. Two ganglionic swellings are present along its course, corresponding to the ganglia of the dorsal roots of the first and second cervical nerves, which are rudimentary in birds. On the sixth day these two swellings have increased somewhat in size and the posterior one, which is the larger, sends out fibers to join a ventral root now present at this level. At some time between the eighth day and the end of incubation, the rudimentary second cervical nerve and both ganglionic swellings disappear.

As previously mentioned, it appears that the motor nucleus of the spinal accessory nerve is essentially a posterior prolongation of the ventral nucleus of the vagus nerve (cf. Fig. 124-B; Fig. 129-F and G). It has been stated that the spinal accessory nerve of the 4.5- to 5-day chick contains a few sensory neuroblasts at the level of the upper part of the spinal cord and that these neuroblasts contribute a few fibers to the uppermost part of the posterior funiculus (*Windle and Orr, 1934*).

### *The Hypoglossal Nerves*

The hypoglossal (cranial XII) nerves innervate the muscles of the base of the tongue and contain special somatic motor elements only. Each nerve leaves the ventral portion of the medulla by a number of roots which unite into a single trunk.

In the chick embryo, neuroblasts have been found in the nucleus of the hypoglossal nerve after 48 hours of incubation. The first axons emerge from the ventral wall of the rhombencephalon about 12 hours later, when 32 somites are present (*Windle and Austin, 1936*). The nerve is well developed in the 78-hour chick, and it is provided throughout its length with cells of Schwann, which appear to be migrating out of the rhomben-



cephalon. At the 96-hour stage, it terminates in a mesoblastic mass on the ventral surface of the pharynx (*Campenhout, 1937a*).

The nucleus of the hypoglossus is a cranial continuation of the ventral somatic motor cell column of the spinal cord, somewhat expanded dorsoventrally at the anterior extremity. On the seventh day of incubation (cf. Fig. 129-F and G), it divides into a dorsomedial and a ventrolateral portion (*Bok, 1915; Eckardt and Elliott, 1935*). It has been said that, in the meantime (on the sixth day), a dorsal nucleus is established between the dorsomedial (ventral) nucleus of the hypoglossus and the dorsal nucleus of the vagus (*Eckardt and Elliott, 1935*). In the 10-day (*Bok, 1915*) or 12-day (*Eckardt and Elliott, 1935*) chick embryo, the rostral portion of the nucleus intermedius becomes united with the dorsal hypoglossal nucleus (*Eckardt and Elliott, 1935*), or, according to the interpretation of Bok (1915), with cells migrated from the dorsomedial (ventral) nucleus.

## THE AUTONOMIC NERVOUS SYSTEM

The second of the two large divisions of the nervous system is the autonomic portion, which regulates the vegetative functions of the body; that is, it innervates the smooth muscles, visceral organs, blood vessels, glands, and so forth. It is composed of general visceral elements.

The autonomic nervous system can itself be subdivided into two portions. There are, first, those general visceral elements which are an integral part of the cerebrospinal nervous system and which effect the continuity of the central nervous system with the second, or peripheral, portion of the autonomic nervous system; the central nervous system is thus given control over the autonomic system. The peripheral autonomic system is comprised of numerous plexuses of nerve fibers and ganglia, usually in close proximity to the organs they innervate.

Unavoidably, many of the general visceral cerebrospinal elements have already been discussed. The cell bodies of general visceral efferent neurones are located in the columns of Terni in the spinal cord and in certain central motor nuclei of the third, seventh, ninth, and tenth cranial nerves; and the cell bodies of the general visceral afferent neurones lie in the spinal ganglia and in certain sensory ganglia of the seventh, ninth, and tenth cranial nerves. The development of autonomic elements, therefore, has been considered in the sections on the brain, the spinal cord, the spinal nerves, and some of the cranial nerves.

The peripheral fibers of the general visceral sensory neurones usually proceed directly to their end organs, regardless of the route taken, and make no functional connections with the cells of autonomic ganglia, many of which ganglia they traverse without interruption. The peripheral fibers of



the centrally lying motor neurones, on the other hand, always make contact with autonomic ganglion cells, and hence are known as preganglionic cells. In distinction, the cells of the autonomic ganglia are often termed postganglionic cells. Their fibers are usually nonmyelinated.

A conspicuous complex of the autonomic nervous system is a paired series of ganglia that lie along the ventrolateral aspect of the vertebral column, one series on each side. The ganglia of each series are connected with each other, with more peripherally lying autonomic ganglia (by means of the visceral nerves), and with the spinal nerves and their roots and ganglia (by means of the rami communicantes). Because of the connections of their cells with elements of the cerebrospinal nervous system, the paravertebral ganglia may be considered as part of the cerebrospinal rather than the peripheral autonomic system, although they are anatomically external to the spinal cord. The ganglia lying at thoracic and upper lumbar levels are segmentally arranged and are usually known as the sympathetic chains, or trunks. The autonomic elements originating from them (and from the cervical, nonsegmental paravertebral ganglia) are termed "sympathetic." In general, they are antagonistic in function to those originating from cranial autonomic ganglia and from the sacral paravertebral ganglia. Elements of the two latter groups are termed "parasympathetic." Most structures innervated by the autonomic system receive both sympathetic and parasympathetic fibers.

The largest peripheral autonomic ganglia and plexuses are located near or within the walls of the thoracic, abdominal, and pelvic viscera. Some are sympathetic and others parasympathetic.

The elements of the paravertebral ganglionic trunks and the peripheral ganglia and plexuses are derived from either or both of two successive waves of cellular migration which are loosely termed the primary and secondary sympathetic trunks. It appears, also, that certain cells of the adrenal medulla are ontogenetically related to the peripheral autonomic nervous system, whose ultimate origin is itself uncertain.

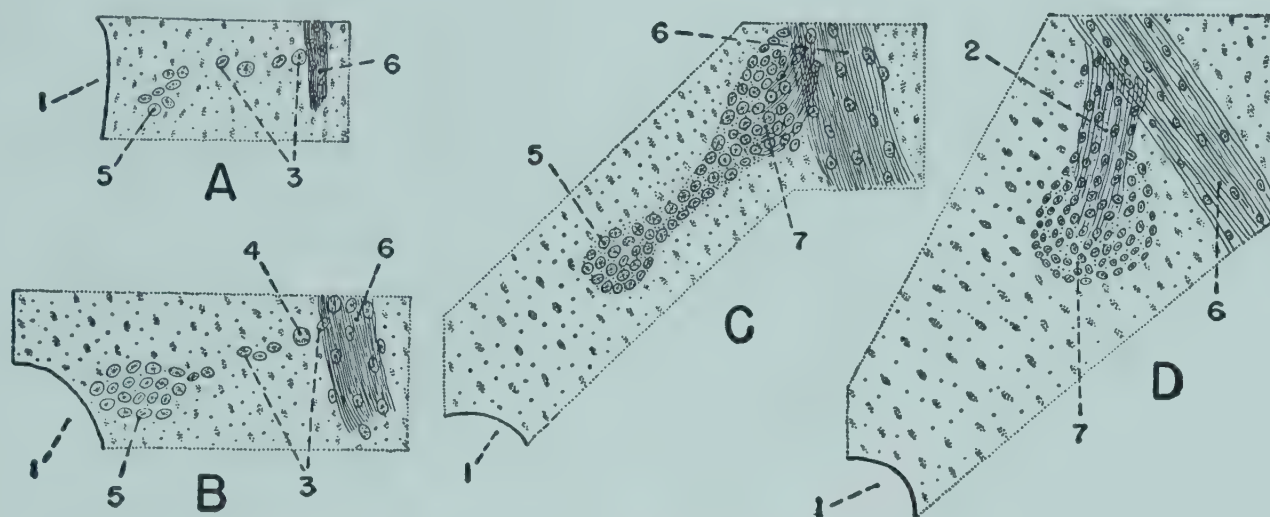
### The Origin of Autonomic Ganglion Cells

The germ layer or the site from which the sympathoblast (the embryonic precursor of the autonomic neurone) differentiates is undetermined. The differentiation of local mesodermal cells into visceral motor neurones is historically the oldest theory (*Remak*, 1847) and has been described in detail by several authors (*Fusari*, 1893; *Tello*, 1924*b*, 1925, 1945; *Levi-Montalcini*, 1947*a*; *Keuning*, 1948). *Tello* (1945) reported the appearance of two cellular trunks adjacent to the aorta between the sixty-eighth and eighty-second hour of incubation of the chick embryo. He stated that these cells aggregate about the segmental arteries and migrate along them to form the sympathetic trunk, which thus obtains, secondarily, its



segmental appearance. The similarity between the sympathoblasts and the endothelium of the aorta suggested to him their common origin.

The origin of autonomic ganglion cells from the spinal ganglia, or from the neural crest via the spinal ganglia, has been described by numerous authors (*Schenk and Birdsall*, 1880; *Onodi*, 1886; *His, Jr.*, 1897; *Müller and Ingvar*, 1923; *Campenhout*, 1931, 1947; *Yntema and Hammond*, 1945; *Hammond and Yntema*, 1947; *Hammond*, 1949; *Wenger*, 1950). Figure 117-A shows a section through the spinal cord (and adjacent tissues) of a 3-day chick embryo with early sympathoblasts reported to have migrated from a spinal ganglion.



**Fig. 130.** Successive stages in the development of the primary and secondary sympathetic trunks of the chick embryo, as seen in transverse sections. (Redrawn after Kuntz, 1910.)

A, the primary sympathetic trunk in the 96-hour embryo ( $\times 100$ ); B, the same in the 105-hour embryo ( $\times 100$ ); C, the primary and secondary sympathetic trunks in the 5-day embryo ( $\times 100$ ); D, the secondary sympathetic trunk in the 6-day embryo ( $\times 50$ ).

1, aorta; 2, communicating ramus; 3, cells migrating into the primary sympathetic trunk; 4, indifferent cell undergoing mitosis; 5, primary sympathetic trunk; 6, spinal nerve; 7, secondary sympathetic trunk.

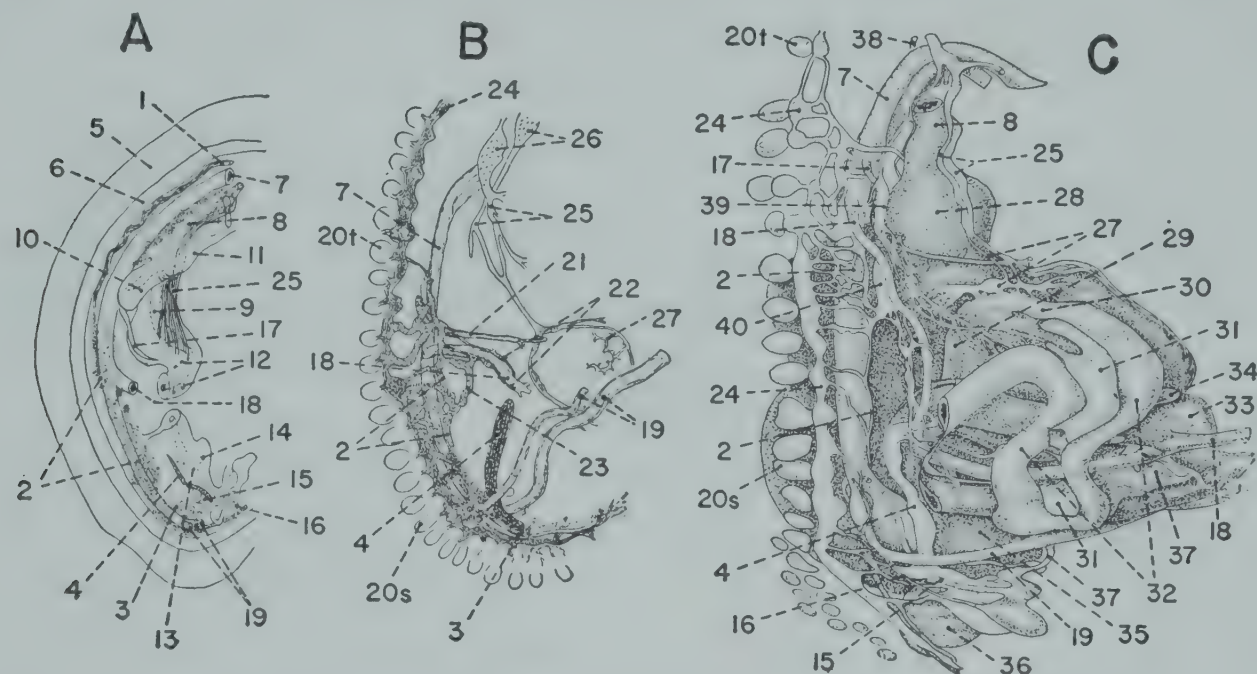
A third group of authors has reported that autonomic ganglion cells are derived primarily, if not entirely, from the neural tube (*Kuntz*, 1910, 1926; *Abel*, 1912; *Jones*, 1937a, 1937b, 1941, 1942; *Brizzee*, 1949a; *Brizzee and Kuntz*, 1950). The thoracolumbar sympathetic ganglion cells, according to these workers, arise in the neural tube in the same region from which the preganglionic cells arise, and migrate via the ventral nerve roots (Fig. 130). *Brizzee* (1949a) reported that the site of origin within the neural tube was the ependyma at the junction of the alar and basal plates. The craniosacral parasympathetic ganglion cells also are considered to arise within the brain stem or ventral cord and migrate via the vagus nerve or the sacral ventral roots. *Kuntz* (1910, 1926, 1945), *Rau and Johnson* (1923), and others have reported that cells also migrate from the spinal ganglia via the dorsal roots into the peripheral autonomic ganglia and



plexuses, but they consider these migrating cells as the primordial supporting cells of the peripheral autonomic nervous system, rather than as ganglion cell anlagen.

### The Primary Sympathetic Trunk

Although the site from which the sympathoblasts actually arise is still undecided, there is general agreement on the time when these cells first become identifiable. Many authors (*His, Jr., 1897; Abel, 1910, 1912;*



**Fig. 131.** The progressive development of the autonomic nervous system in the chick, reconstructed from photographs of frontally sectioned embryos. (Redrawn with modifications after *His, Jr., 1897.*)

A, at 4 days; B, at 6 days; C, at 10 days. All  $\times 5$ .

1, primary sympathetic trunk; 2, preaortic plexus; 3, pelvic plexus; 4, ganglion of Remak; 5, spinal cord; 6, notochord; 7, dorsal aorta; 8, esophagus; 9, stomach; 10, lung bud; 11, trachea; 12, liver and pancreatic diverticulæ; 13, large intestine; 14, caecum; 15, mesonephric duct; 16, Müllerian duct; 17, coeliac artery; 18, omphalomesenteric artery; 19, umbilical artery; 20t, first thoracic spinal ganglion; 20s, first sacral spinal ganglion; 21, coeliac nerve; 22, mesenteric nerve; 23, splanchnic nerve and plexus; 24, secondary sympathetic trunk; 25, vagus nerves; 26, nodose ganglia; 27, vagal plexus in wall of stomach; 28, proventriculus; 29, gizzard; 30, pylorus; 31, pancreas; 32, small intestine; 33, transverse colon; 34, tip of caecum; 35, rectum; 36, cloaca; 37, allantoic duct; 38, recurrent nerve; 39, coeliac ganglion; 40, mesenteric ganglion.

*Müller and Ingvar, 1923; Uchida, 1927; Campenhout, 1931; Jones, 1937b*) agree that the migration of sympathoblasts and indifferent cells into the region dorsolateral to the aorta and dorsal to the carotid arteries becomes evident in the chick during the third day of incubation. Ramon (1908a) has observed cells which he identified as sympathoblasts in this region as early as the 52-hour stage, and Tello (1925, 1945), who believed that sympathoblasts differentiate *in situ*, has described the primary trunk as extending from the fifth cervical segment to the twenty-fourth or twenty-fifth somite after 68 to 76 hours of incubation. By the eighty-fourth hour,



it has increased in size and has extended ahead of the fifth cervical segment to the level of the vagus and glossopharyngeal nerves (Tello, 1925). The trunk, however, is not well established until about 4.5 days of incubation (Rabl, 1891; His, Jr., 1897; Abel, 1910, 1912; Kuntz, 1910; Müller and Ingvar, 1923; Jones, 1937b). Figures 131-A and 132-A show the extent of the primary trunk in the 4-day chick embryo. The primary trunk also appears in Fig. 117-B and C, which represent sections of the spinal cord of the 4- to 4.5-day chick embryo, and in Fig. 132-B,

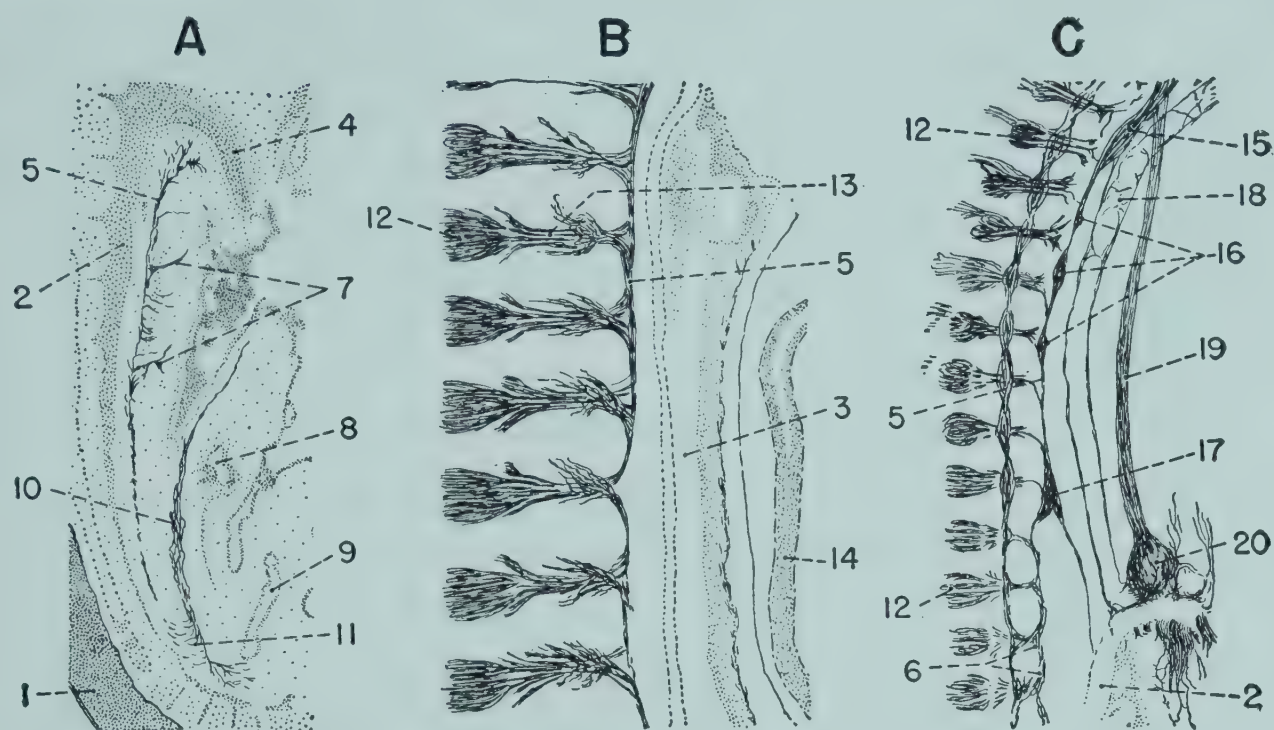


Fig. 132. The development of the primary and secondary sympathetic trunks in the chick embryo, as seen in longitudinal sections. (Redrawn with modifications A, after Tello, 1924b; B and C, after Tello, 1925.)

A, the primary sympathetic trunk and the ganglion of Remak in a 4-day embryo ( $\times 20$ ); B, the primary sympathetic trunk and its relation to the spinal ganglia in a 5-day embryo ( $\times 20$ ); C, the primary and secondary sympathetic trunks, the cervical ganglia, and the vagus nerve in a 6-day embryo ( $\times 10$ ).

1, spinal cord; 2, dorsal aorta; 3, carotid artery; 4, omphalomesenteric artery; 5, primary sympathetic trunk; 6, secondary sympathetic trunk; 7, branches into suprarenal region; 8, large intestine; 9, cloaca; 10, ganglion of Remak; 11, pelvic plexus; 12, spinal ganglion; 13, ventral spinal root; 14, esophagus; 15, superior cervical ganglion; 16, intermediate cervical ganglion; 17, inferior cervical ganglion; 18, carotid plexus; 19, vagus nerve; 20, vagal thoracic plexus.

which shows the primary trunk in relation to the spinal ganglia of the 5-day chick embryo. The early development of the primary sympathetic trunk in the sparrow (*Passer domesticus*), as reported by Rau and Johnson (1923), is similar to the same stage in the chick.

During the chick's fourth day of incubation, there is a migration of sympathoblasts toward the ventrolateral aspect of the aorta from the region of the suprarenal bodies caudad (His, Jr., 1897; Kuntz, 1910; Abel, 1912; Tello, 1925). This migration forms the aortic, or preaortic, plexus (cf. Fig. 131-A and B). During the fifth day, the migration of cells



ventralward continues, and some cells invade the mesenteries. In the region of the suprarenal body a well-defined migration of cells occurs (*Rabl, 1891; His, Jr., 1897; Kuntz, 1910*), to form the so-called "Geschlechtsnerven" of Remak. The migration of cells from the primary sympathetic trunk is completed by the sixth day of incubation. The primary trunk in the abdominal region is then exhausted, but it persists in the upper thoracic and cervical regions as the irregular cervical sympathetic trunk, including the inferior, intermediate, and superior cervical ganglia (Fig. 132-C). The aortic plexus and its derivatives, a large part of the enteric plexuses of the intestine, the adrenal medulla, and other abdominal visceral ganglia and plexuses are thus derived from the primary sympathetic trunk. It has been suggested that the secondary sympathetic trunk also is derived from the primary sympathetic trunk (*Tello, 1925, 1945*). The peripheral autonomic system in the chick at 6 days is seen in Fig. 131-B and Fig. 132-C.

### The Secondary Sympathetic Trunk

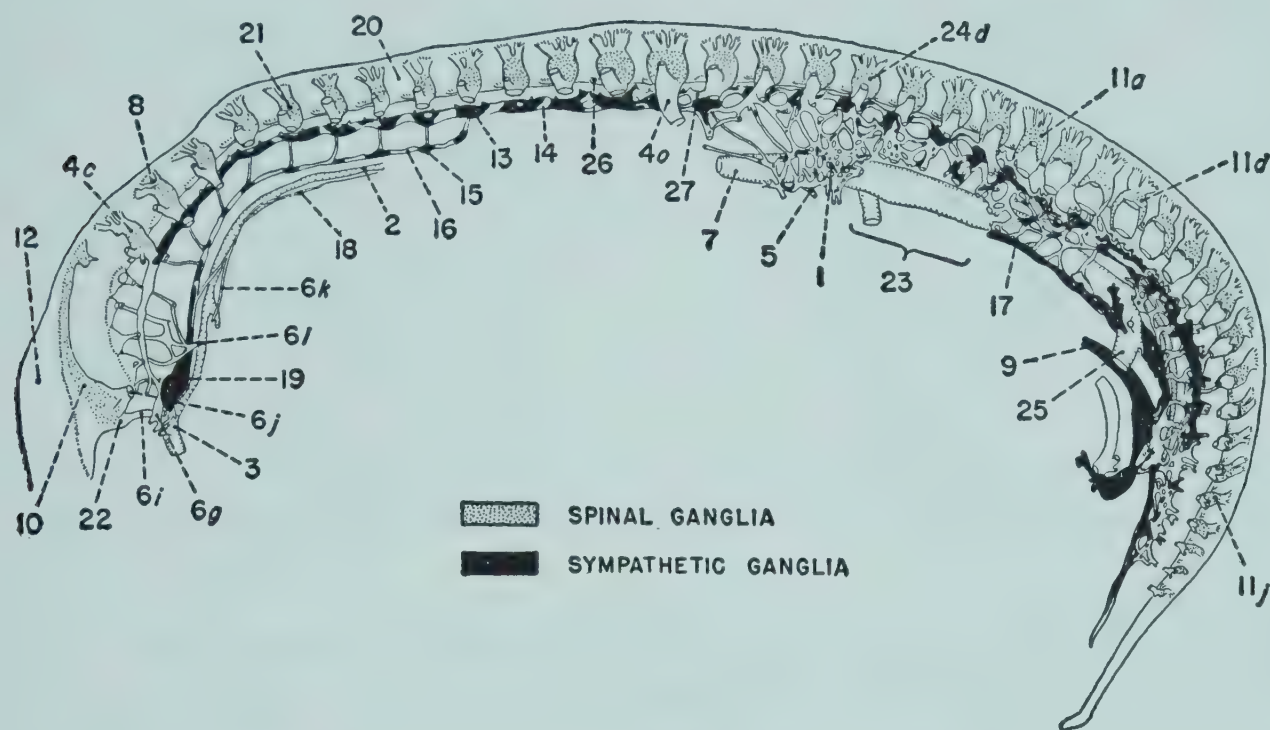
As the primary sympathetic trunk begins to disappear, a new migration of cells begins to form a second series of segmental ganglia (cf. Fig. 130-C and D) ventromedial to the spinal nerve roots near the point where the dorsal and ventral roots merge. This process begins in the chick on the sixth day of incubation (*His, Jr., 1897; Kuntz, 1910; Abel, 1912; Tello, 1925*). The ganglia are at first independent of each other, but rapidly become interconnected (*Kuntz, 1910*), as Fig. 131-B shows. Originally thirty ganglia are formed in each secondary sympathetic trunk of the chick, one opposite the jugular ganglion of the vagus and one opposite each spinal nerve down to the twenty-ninth. Each of these ganglia soon acquires connections with (1) the central nervous system, (2) peripherally with the original primary sympathetic trunk and its derivatives, and (3) with the ganglia cranial and caudal to it. Abel (1912) has suggested that the secondary sympathetic trunk ganglia contribute some cells into the more peripheral ganglia and plexuses already established by the primary sympathetic trunk.

A few authors have proposed that the ganglia of the secondary sympathetic trunk are derived from the primary sympathetic trunk (*Tello, 1925, 1945; Rau and Johnson, 1923*), but the majority of authors have considered it a new migration from the spinal cord or from the neural crest and spinal ganglia. Staudacher (1940) suggested that the preganglionic fibers exerted an organizing influence on the development of the secondary trunk ganglia. This has been denied by Hammond (1949). Simmler (1949) has reported that peripheral factors do not influence the early phase of development of these ganglia in the brachial region, but that, in later



stages, the ganglia respond in the same manner as somatic centers to reduction of their peripheral fields.

The ganglionic trunks of the 6-day and 10-day chick embryo are seen in Fig. 131-B and C, respectively, and that of the 8-day chick in Fig. 133, which shows the relationship of the spinal nerve roots and ganglia to the paravertebral autonomic ganglia.



**Fig. 133.** The spinal cord, the spinal nerves, and the autonomic trunk and ganglia of an 8-day chick embryo;  $\times 8$ . (Redrawn with modifications after Yntema and Hammond, 1945, and Hammond and Yntema, 1947.)

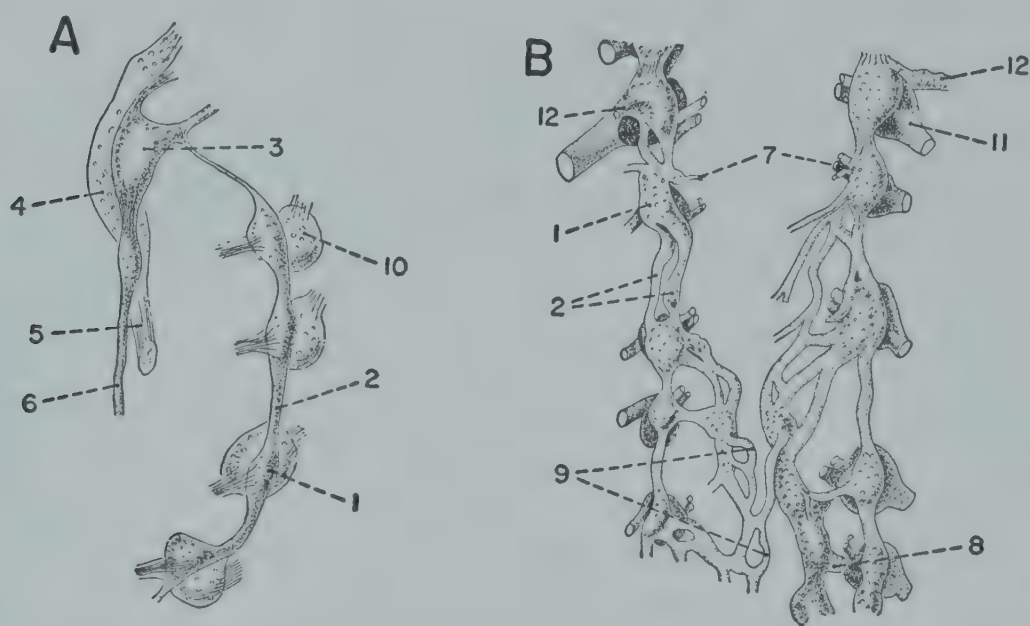
1, aortic plexus; 2, common carotid artery; 3, internal carotid artery; 4c, third cervical nerve; 4o, fifteenth cervical nerve; 5, coeliac artery; 6g, rami to VII cranial nerve; 6i, IX cranial nerve; 6j, X cranial nerve; 6k, branch from IX cranial nerve; 6l, XII cranial nerve; 7, dorsal aorta; 8, dorsal root; 9, ganglion of Remak; 10, jugular ganglion; 11a, first lumbar nerve; 11d, fourth lumbar nerve; 11j, tenth sacral nerve; 12, medulla oblongata; 13, paravertebral ganglion; 14, paravertebral trunk; 15, postcarotid ganglion; 16, postcarotid trunk; 17, preaortic column; 18, precarotid nerve; 19, superior cervical ganglion; 20, spinal cord; 21, spinal ganglion; 22, superior ganglion of cranial IX nerve; 23, suprarenal area; 24d, fourth thoracic ganglion; 25, umbilical artery; 26, ventral root; 27, white communicating ramus. (Letters following numbers indicate alphabetically the consecutive order of the structures.)

### The Superior, Intermediate, and Inferior Cervical Ganglia

In the 76-hour chick embryo, the primary sympathetic trunk extends ahead of the fifth somite as a fine commissure and reaches as far as the nuclei of the vagus and glossopharyngeal nerves (*Tello, 1925*). As the primary sympathetic trunk disappears during the period from the sixth to the eighth day, this portion of it remains and becomes a part of the definitive paravertebral ganglionic trunk. A ganglion develops in the rostral end of this chain as the superior cervical ganglion, which gives two branches rostrally into the head and a single branch posteriorly,



the latter accompanying the vagus nerve to the heart (*His, Jr., 1893*) (see Fig. 134-A). Below the superior cervical ganglion are two or three smaller intermediate cervical ganglia, and at the level of the thirteenth cervical segment a small inferior cervical ganglion (*Tello, 1925*). Figure 132-C shows these ganglia as they appear in the 6-day chick embryo.



**Fig. 134.** The cervical and upper thoracic sympathetic chain of the 10-day chick embryo. (Redrawn with modifications after *His, Jr., 1893*.)

A, the upper portion of the cervical sympathetic chain and the superior cervical ganglion; B, the upper portion of the thoracic sympathetic trunks, the coeliac plexus, and the roots of the sympathetic cardiac nerves. Both  $\times 8$ .

1, sympathetic ganglion; 2, sympathetic trunk; 3, superior cervical ganglion; 4, nodose ganglion of cranial X nerve; 5, vagus (cranial X) nerve; 6, sympathetic nerve to the heart along the carotid artery; 7, root of sympathetic cardiac nerves; 8, renal nerve; 9, coeliac plexus; 10, third cervical spinal ganglion; 11, first thoracic spinal ganglion; 12, sympathetic ramus to vena cava.

### The Peripheral Autonomic Ganglia and Plexuses

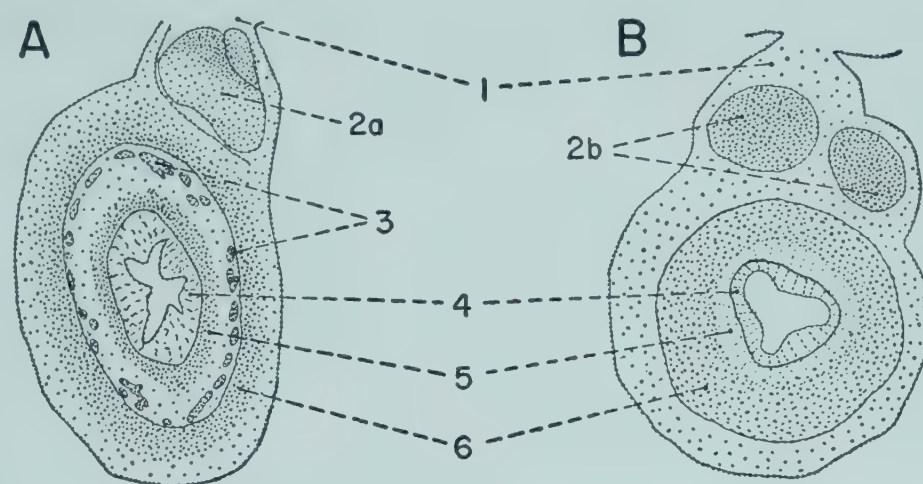
Some of the more peripheral portions of the autonomic nervous system are recognized as being derivatives of the primary sympathetic trunk and thus are sympathetic in nature. Others are recognized as being derived from the vagus nerve or from the sacral spinal nerves and thus are parasympathetic in nature. The origin of a few of the ganglia and plexuses is still controversial.

#### *The Ganglion of Remak*

Remak (1847) described an unpaired nerve lying in the mesorectum, mesocolon, and mesentery of the chicken. After 8 days of incubation it extended as far as the "umbilical remnant" and after 10 days it had reached the caudal limits of the duodenum. Onodi (1886) identified the structure in the duck (*Anas platyrhynchos*), but described it as a double nerve (Fig. 135-B). *His, Jr., (1897)* reported the ganglion of Remak to



be well developed in the 4-day chick embryo, but could observe no connection of the nerve with any other part of the autonomic system. On the fifth day it extended from the cloacal region to the caecal anlagen and on the sixth day received a migration of cells from the sacral autonomic regions via the pelvic plexus (*His, Jr., 1897*). Abel (1912)



**Fig. 135.** The ganglion of Remak in the chick embryo (*Gallus gallus*) embryo and in the duck (*Anas platyrhynchos*) embryo. (Redrawn with modifications A, after Jones, 1942; B, after Onodi, 1886.)

A, a cross section of the rectum of an 8-day chick embryo, transecting the ganglion of Remak and the enteric plexuses in the gut wall ( $\times 50$ ); B, a cross section of the gut of a 6-day duck embryo, showing a paired ganglion of Remak ( $\times 100$ ).

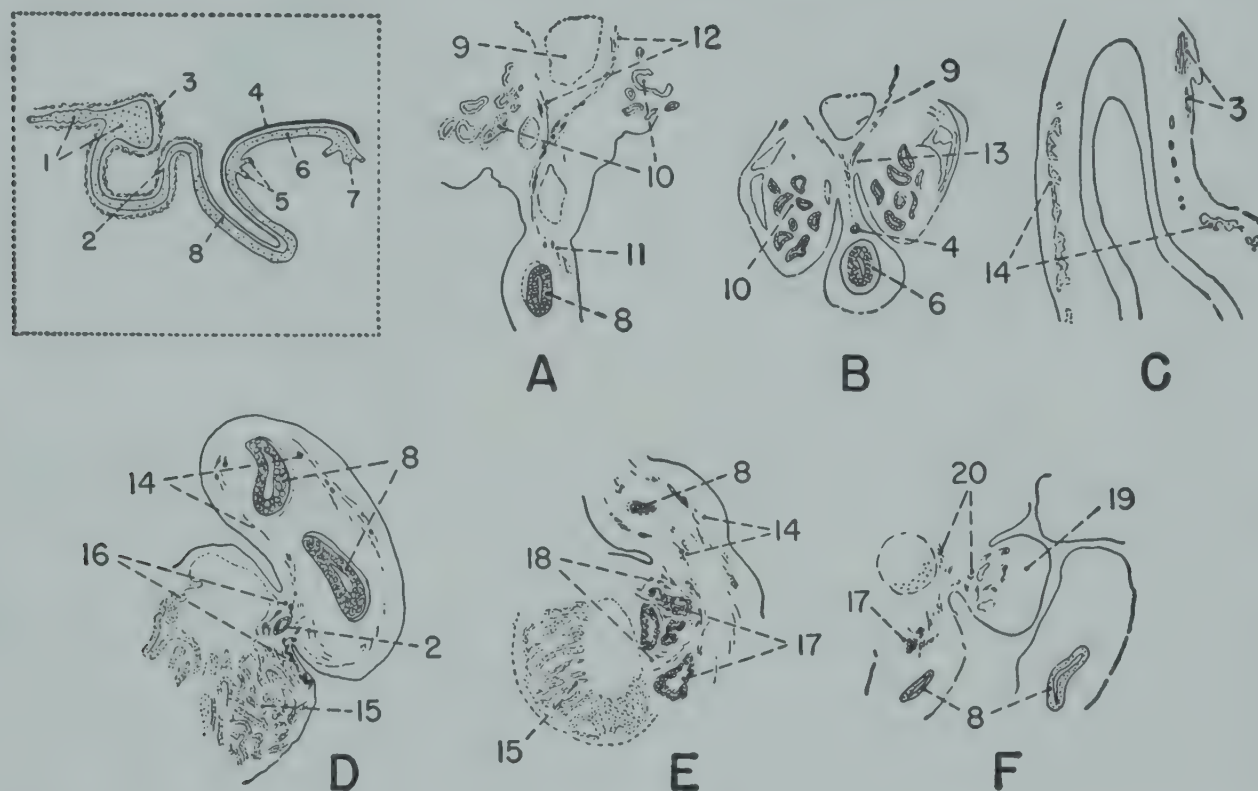
1, mesorectum; 2a, single ganglion of Remak; 2b, paired ganglion of Remak; 3, developing enteric plexus; 4, mucosa; 5, submucosa; 6, muscularis.

identified the ganglion and considered that it was derived from the primary sympathetic trunk, as suggested by Kuntz (1910), who proposed, in addition, that it was related to the oviparous habit of birds. Campenhout (1931) described the ganglion of Remak as composed of the visceral branches of five pairs of sacral autonomic ganglia. Jones (1942) reported that the ganglion developed during the fourth day of incubation from the sacral nerves and the caudal primary sympathetic trunk and that cells migrated from it into the intestinal walls to form a part of the enteric plexuses. Yntema and Hammond (1955) investigating the chick embryonic origin of the neuroblasts of the pelvic plexus and the nerve of Remak suggest the neuroblasts arise from the lumbosacral neural crest and form a part of the sacral parasympathetic system. The authors indicate the nerve of Remak is an ascending structure whose branches to the intestine contain postganglionic fibers. Figure 136-B and Fig. 135-A show the position of the ganglion in the mesorectum of the chick at 6 and 8 days, respectively, and the small diagram inserted in Fig. 136 shows the relationship of the ganglion to the other parasympathetic nerves of the intestines at 6 days. The relationship of this ganglion to other peripheral autonomic structures at 4, 6, and 10 days of incubation may be seen in Fig. 132-A; Fig. 131-A, B, and C.



*The Coeliac, "Hypogastric," and Pelvic Ganglia and Plexuses*

The aortic, or prevertebral, plexus is derived from the primary sympathetic chain during the fifth and sixth days of incubation, as already mentioned. Subsequently, this plexus becomes enlarged in three regions; the resulting ganglia and plexuses are called the coeliac, pelvic, and "hypogastric" plexuses, the last of which is represented in birds by the ganglion



**Fig. 136.** The development of several of the autonomic ganglia and plexuses in the abdominal region of the chick embryo. (Redrawn with modifications A, B, D, E, F, after Abel, 1912; C, after Jones, 1942.)

A, the mesenteric plexus in the 6-day embryo; B, the ganglion of Remak in the 6-day embryo; C, the enteric plexus in the wall of the gizzard of a 7-day embryo; D, the hepatic plexus in the 6-day embryo; E, the pancreatic plexus in the 7-day embryo; F, the splenic plexus in the 7-day embryo. All  $\times 25$ .

*Insert:* a schema of the innervation of the gut of the 6-day embryo. (After Jones, 1942.)

1, stomach; 2, bile duct; 3, vagal nerve and plexus; 4, ganglion of Remak; 5, caeca; 6, rectum; 7, cloaca; 8, small intestine; 9, dorsal aorta; 10, mesonephric kidney; 11, mesenteric plexus; 12, preaortic plexus developing from the primary sympathetic trunk; 13, pelvic plexus; 14, enteric plexus; 15, liver; 16, ganglion cells of the hepatic plexus migrating into the liver; 17, pancreas; 18, nerve plexus developing in the pancreas; 19, spleen; 20, ganglion cells migrating into the spleen to form a plexus.

of Remak, according to Kuntz (1910). The coeliac is the largest of these plexuses and surrounds the coeliac artery at the point where it arises from the aorta. From this plexus spring sprouts to the suprarenal gland, pro- and mesonephroi, and the gonads; and it also builds the splanchnic or mesenteric plexuses (*His, Jr., 1897*). In the 6-day chick embryo, *His, Jr., (1897)* observed three large ganglion-free nerves leaving the coeliac plexus: (1) a nerve following the coeliac artery to the stomach,



liver, and duodenum; (2) a nerve to the pancreas via the mesenteries; and (3) a nerve into the mesenteric plexus. Also, at 6 days, the secondary sympathetic trunk is in contact with the coeliac and pelvic plexuses from the thirteenth segment caudad. The cords from the thirteenth to the twentieth segments form the anlagen of the splanchnic nerves. The 10-day chick has a well-developed coeliac plexus (Fig. 134-B), and the right vagus nerve joins it at this time (*His, Jr., 1897*). The pattern of the peripheral autonomic ganglia and plexuses closely resembles the adult pattern, as may be seen in Fig. 131-C.

### *The Enteric and Mesenteric Ganglia and Plexuses*

The enteric ganglia in the walls of the esophagus and stomach are generally considered to be derived from cells which migrate along the course of the vagus nerve (*Meiklejohn, 1908; Kuntz, 1910; Abel, 1912; Yntema and Hammond, 1945; Jones, 1942*). Keuning (1944, 1948) suggested their origin *in situ* from mesenchyme cells, after observing their differentiation in cultures of the chick's esophagus in the absence of vagus fibers. Campenhout (1931, 1932, 1933) stated that the gastric portion develops independently of the vagus from the prevertebral sympathetic plexus and receives vagal components only secondarily. Kuntz (1934) pointed out, however, that the prevertebral plexuses do not develop in the chick embryo until after the appearance of sympathoblasts in the gastric wall, over which the vagus has already spread at this time (the ninety-sixth hour). The enteric plexuses of the upper intestine have been said to be derived from the vagus (*Kuntz, 1910; Abel, 1912; Jones, 1942*) and from the prevertebral plexus (*Campenhout, 1931, 1933; Dereymaeker, 1943*). The enteric plexus in the lower intestine is derived initially from the ganglion of Remak (*His, Jr., 1897*).

Kuntz (1910) observed the migration of cells along the vagus, which surrounds the esophagus at about 5.5 days of incubation (Fig. 137-A and B). By the seventh and eighth days, there is a well-established plexus in the esophageal wall. After 4.5 days of incubation, the vagus has reached the stomach; and after 5 days, an extensive plexus has appeared (*Abel, 1912*). During the seventh day the vagus extends into the upper intestines, and two zones are rapidly established: (1) an inner submucous (Meissner's) plexus, and (2) an outer myenteric (Auerbach's) plexus (*His, Jr., 1897; Abel, 1912*). Figure 131-B and C, Fig. 135-A, and Fig. 136-C and D show various aspects of the development of the enteric plexuses.

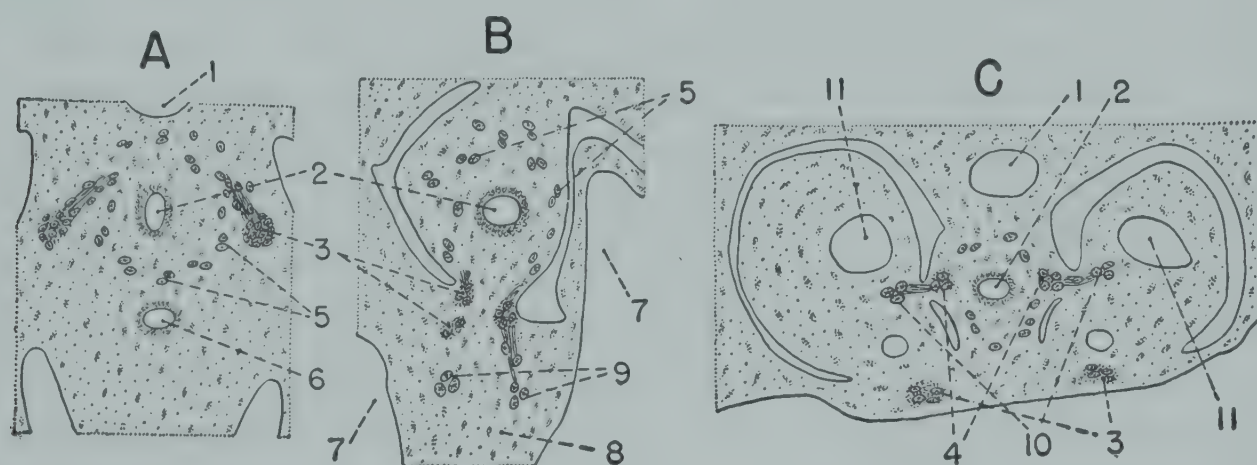
The mesenteric plexuses develop from the prevertebral plexus during the fifth day (*His, Jr., 1897; Abel, 1912; Campenhout, 1933*). Figure 136-A shows the development of this plexus in the 6-day chick embryo, and Fig. 131-B and C show the plexus and its relation to other parts of the autonomic nervous system of the chick embryo at 6 and 10 days.



By the tenth day the mesenteric plexus connects caudally with the ganglion of Remak in the region of the last loop of the small intestine (*His, Jr., 1897*).

### *Other Ganglia and Plexuses of the Abdominal Viscera*

*His, Jr., (1897)* described the innervation of the chick's liver from the coeliac ganglion after the fifth day of incubation. By 6 days, the liver is a large organ, and nerve cells are seen migrating from the duodenal plexus along the common bile duct into the liver (Fig. 136-D), forming a delicate plexus in the porta (*Abel, 1912*). By the seventh day, the plexus is extensive and spreads between the trabeculae of the liver and, as a fine plexus, over the developing gall bladder (*Abel, 1912*).



**Fig. 137.** The development of the esophageal, pulmonary, and cardiac plexuses in the chick embryo. (Redrawn with modifications after *Kuntz, 1910*.)

A, cross section through the esophagus and vagal nerves of a 6-day embryo, showing the early development of the esophageal plexus; B, cross section through the esophagus of a 6-day embryo, showing the anlagen of the esophageal and cardiac plexuses; C, cross section through the prospective lung region of a 7-day embryo, showing the early development of the pulmonary plexus. All  $\times 40$ .

1, aorta; 2, esophagus; 3, vagus nerves; 4, branches of the vagi; 5, anlagen of the myenteric and submucous plexuses of the esophagus; 6, trachea; 7, atrial cavity; 8, atrial septum; 9, anlagen of the cardiac plexus; 10, anlagen of the pulmonary plexuses; 11, bronchus.

The innervation of the pancreas by a small branch of the middle mesenteric nerve from the coeliac ganglion has been described by *His, Jr., (1897)* as taking place about on the fifth day. This innervation has been considered to be, at least in part, vagal (*His, Jr., 1897*), mostly vagal (*Abel, 1912*), and entirely vagal initially (*Kuntz, 1910; Jones, 1942*); but *Campehouth (1933)* stated that it was entirely sympathetic. At 6 days the dorsal and ventral pancreatic diverticuli are well established, and fibers and cells are seen migrating into them from the duodenal plexus (Fig. 136-E) along the pancreatic ducts. This plexus is well established about the glandular tissue of the pancreas by the seventh day (*Abel, 1912*).

The spleen can be identified in the chick at 6 days, but no autonomic



cells migrate into it until the seventh day. These cells migrate from the sympathetic trunk and may be seen in Fig. 136-F (Abel, 1912).

The innervation of the gonads is derived from the sympathetic trunk (Abel, 1912; Campenhout, 1931). During the sixth day, delicate outgrowths appear from a small ganglion lying between the germinal ridge and the body wall and pass toward, but not into, the gonads. At 7 days, these fibers form a delicate plexus just dorsal to the gonad (Abel, 1912).

### *The Cardiac and Pulmonary Ganglia and Plexuses*

These two ganglia and plexuses develop from cells which accompany the vagus nerve and which are derived either from the hindbrain (Kuntz, 1910; Abel, 1912; Jones, 1942) or from the occipital and upper cervical neural crest (Campenhout, 1937a). The vagus nerve appears at about 38 to 39 hours in the chick (Campenhout, 1937a). At 4.5 days, a fine streaming of cells from the vagi along the course of the aortic arches toward the bulbus arteriosus has been observed by Abel (1912). At 5 days, cells may be traced into the atrial septum (Kuntz, 1910) from the vagi at the level of the bifurcation of the trachea (Fig. 137-B). At 6 days, the plexuses about aortic arches III, IV, and VI are well developed (Abel, 1912). From the right vagus nerve a small twig lies on the lateral surface of aortic arch IV just proximal to the origin of the innominate artery (His, Jr., 1893). This twig divides, giving a small bundle into the angle between the subclavian and carotid arteries which ends in a small ganglion. The second branch passes into the septum of the bulbus arteriosus and ends there in a small ganglion (His, Jr., 1893). The left vagus nerve gives two small nerves from its recurrent branch into the septum of the bulbus arteriosus via the left pulmonary trunk. These fibers also terminate in a small ganglion (His, Jr., 1893). At 7 days, the "bulbus nerve" of His is formed by the fusion of the bundles at the junction of the arteries with the ventricular wall (Abel, 1912), and a plexus forms in the posterior interventricular groove. At 8 days, an auricular plexus has developed from a new bundle derived from the vagus and in part from the bulbus nerve. The right bulbus nerve branches, forming a T-shaped terminus in the auriculo-ventricular groove, and is connected with the left bulbus nerve (His, Jr., 1893). There is also a connection with the pulmonary plexus (His, Jr., 1893; Abel, 1912).

At 9 days, the plexus in the auriculo-ventricular groove is well established (Abel, 1912). At 10 days, two small coronary nerves without any ganglion proper have developed from this plexus (His, Jr., 1893). The innervation of the heart by a small branch from the superior cervical ganglion may also be identified at 10 days (His, Jr., 1893, 1897) and may be seen in Fig. 134-A.

The pulmonary plexus develops from the vagi during the fifth day



(Abel, 1912) and at 7 days may be traced into the region of the lung bud (Kuntz, 1910). The early development of this plexus may be seen in Fig. 137-C. The pulmonary plexus contributes a small bundle into the cardiac plexus at about 8 days of incubation in the chick.

### *The Cranial Autonomic Ganglia*

In the cephalic region are a number of autonomic plexuses and ganglia. The plexuses are regarded as extensions of the paravertebral trunk and have not been studied in the developing chick. They include the plexuses of the internal carotid, external carotid, and common carotid arteries, and the cavernous and tympanic plexuses. The cranial ganglia are situated in relation to several of the cranial nerves and are generally considered to be parasympathetic in nature. They are the ciliary, sphenopalatine, otic, submaxillary, submandibular, and lingual ganglia. Few investigations have been made on their initial development, possibly because of the complexity of the early morphogenesis of the cranial region and the controversial nature of the primary origin of the ganglionic neuroblasts.

Kuntz (1945) has suggested that the cranial autonomic ganglia develop in the chick in much the same manner as in the mammal. The sphenopalatine ganglia possibly develop from cells which migrate from the geniculate ganglia along the greater superficial petrosal nerves, and the semilunar ganglia may contribute cells via the maxillary nerves and their sphenopalatine rami. The otic ganglia, which develop at the tip of the lesser superficial petrosal nerves, are perhaps formed by cells which have migrated from (1) the glossopharyngeal nerves via the tympanic rami, (2) the semilunar ganglia, or (3) both of these sources. The submaxillary ganglia may result from the accumulation of cells in the paths of the lingual nerves migrating from the semilunar ganglia. Later the chorda tympani may contribute cells of facial origin. The sublingual ganglia may arise much as do the submaxillary ganglia; and the lingual ganglia, small ganglia at the back of the tongue associated with the glossopharyngeal nerves, may be derived from cells which migrate along these nerves. (Figure 123-C shows the nerves mentioned here as being involved in the development of the cranial autonomic ganglia.)

**The Origin of the Ciliary Ganglia.** There are several opinions regarding the derivation of the ciliary ganglia in the chick. The earliest theory is that of Marshall (1878), who believed that ganglionic neuroblasts migrated out from the midbrain along the oculomotor nerve. Carpenter (1906) suggested that some of the satellite cells along the course of the early nerve give rise to cells of Schwann, whereas the remainder differentiate into large cells making up the ventral portion of each ganglion. He proposed that the small cells of the dorsal portion migrate along a ramus of the ophthalmic branch of the trigeminal nerve from the ophthal-



mic lobe of the trigeminal (semilunar or Gasserian) ganglion. Campenhout (1937a) similarly expressed the belief that the ganglion forms as the result of the separation of the anterior part of the ophthalmic lobe of the trigeminal ganglion, which then grows out as a fibrillo-ganglionic strand. He made no mention, however, of any contribution to the ganglion by way of the oculomotor nerve. According to Tello (1923), the ganglionic neuroblasts differentiate *in situ* from mesenchyme.

Experimental extirpation of the proposed sources of the cells of the ciliary ganglion from chick embryos less than 40 hours old (usually of 8 to 14 somites) has demonstrated fairly definitely that the semilunar ganglion of the trigeminal nerve plays no part in the formation of the ciliary ganglion (Amprino, 1942; Jones, 1945; Levi-Montalcini and Amprino, 1946, 1947). Similarly, removal of the midbrain, although preventing the appearance of the oculomotor nerve, does not affect the development of the ganglion (Jones, 1945; Levi-Montalcini and Amprino, 1946, 1947). Excision of the telencephalon has given conflicting results. Jones (1945) reported that the ciliary ganglion failed to develop after extirpation of the mesencephalon and the portion of the telencephalon lying between the optic stalks. He suggested that the cells of the ciliary ganglion migrate from the forebrain region to the mesencephalon (most probably), and thence along the oculomotor nerves. These results could not be confirmed by Levi-Montalcini and Amprino (1947), who, by the process of elimination, found that the only portion of the head whose removal affected the development of the ciliary ganglion was the mesenchyme in the mesencephalic region of the 6- to 12-somite embryo. The ciliary ganglion degenerated after the tenth incubation day in the absence of the mesencephalon, but this occurrence was attributed to lack of functional stimuli from preganglionic centers in the midbrain (Levi-Montalcini, 1947b). The inference was made that the ganglion is derived from mesectoderm of neural crest origin, a conclusion which supported the view of Tello (1923).

### The Origin of the Suprarenal Medulla

It is generally accepted that the chromaffin cells of the adrenal medulla are derived from the sympathetic nervous system (Hammond and Yntema, 1947). A ventral extension from the primary sympathetic trunk (Fig. 138-A) toward the cortical bodies (the "Geschlechtsnerven" of Remak) has been reported in the 4-day chick embryo (Rabl, 1891; His, Jr., 1897; Willier, 1929, 1930; Brauer, 1932). Fusari (1893) observed this anlage as early as the seventy-eighth hour and stated that it became lodged on the dorsomedial surface of the cortical body by the nineteenth hour. Minervini (1904) and Abel (1912) did not identify this ganglion in the chick until the fifth or sixth day. Soulié (1903) identified it in the zebra parakeet (*Melopsittacus undulatus*) at 94 hours. Willier (1929) suggested that the



secondary sympathetic trunk, when it appears, contributes some additional cells to the developing suprarenal ganglion and to the medulla.

Penetration of the primordial medullary cells (the chromaffin cells) into the cortical body (Fig. 138-B) and the establishment of a medulla begins in the chick at about 7 days of incubation (Poll, 1906; Abel, 1912; Brauer, 1932), or possibly as early as 6.5 days (Goormaghtigh, 1921), but not in the zebra parakeet (*Melopsittacus undulatus*) until about 15 days (Soulié, 1903). Hoffmann (1892) has reported that the suprarenal medulla has about the same developmental course in marsh birds as in the chick. In the 7-day chick, three types of cells have been identified by Brauer (1932)

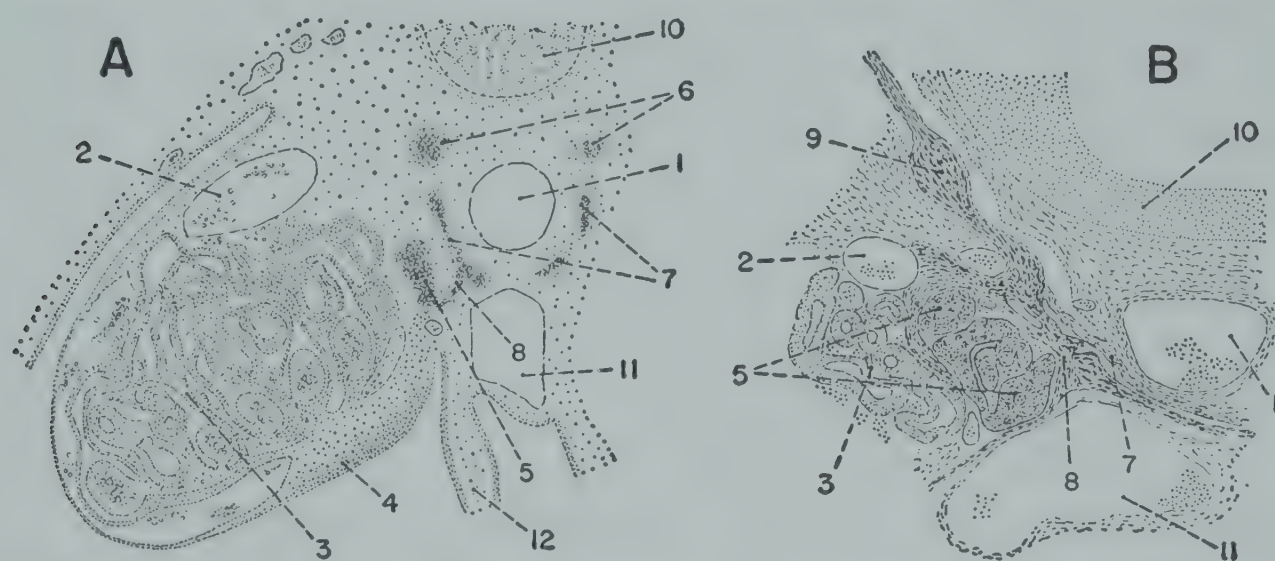


Fig. 138. The development of the suprarenal plexus and medulla in the embryo of the European black-tailed godwit, *Limosa limosa*. (Redrawn with modifications after Hoffmann, 1892.)

A, a cross section through a young embryo, corresponding approximately to a 4.5-day chick embryo, showing the primary sympathetic trunk, the preaortic plexus, and the migration of ganglion cells toward the cortical body; B, a cross section through an older embryo, showing the sympathetic ganglion and plexus on the dorsomedial surface of the cortical body and the invasion of that structure by sympathetic cells. Both  $\times 25$ .

1, dorsal aorta; 2, posterior cardinal vein; 3, mesonephros; 4, germinal ridge; 5, cortical body; 6, primary sympathetic trunk; 7, preaortic plexus; 8, suprarenal plexus; 9, sympathetic ganglion and nerve; 10, notochord; 11, inferior vena cava; 12, root of mesentery.

as involved in this process: (1) sympathetic ganglion cells on the surface of the suprarenal body, (2) sympathetic ganglion cells whose fibers penetrate the gland, and (3) chromaffin cells migrating into the gland and forming the medulla. The latter cells are not readily distinguished in the chick until 7.5 to 8 days of incubation (Brauer, 1932).

### THE NONNERVOUS ELEMENTS OF THE NERVOUS SYSTEM

The nervous system contains many elements which are not neural in nature. Some of these elements provide a framework for the nervous tissues and others permit the nervous system to receive nourishment from the blood stream.



### The Supporting Tissues

The supporting tissues are the neuroglia, ependyma, and microglia; the sheath and capsular cells; and the myelin sheath. The ependymal cells, lining the central canal, are formed from spongioblasts which differentiate from the neural epithelium and retain their original position. Many spongioblasts, on the other hand, migrate into the mantle and marginal layers of the neural tube and differentiate either into astrocytes or into oligodendroglia, the two chief neuroglial elements. Neuroglial cells were first described by Golgi (1886) and their presence in birds was confirmed

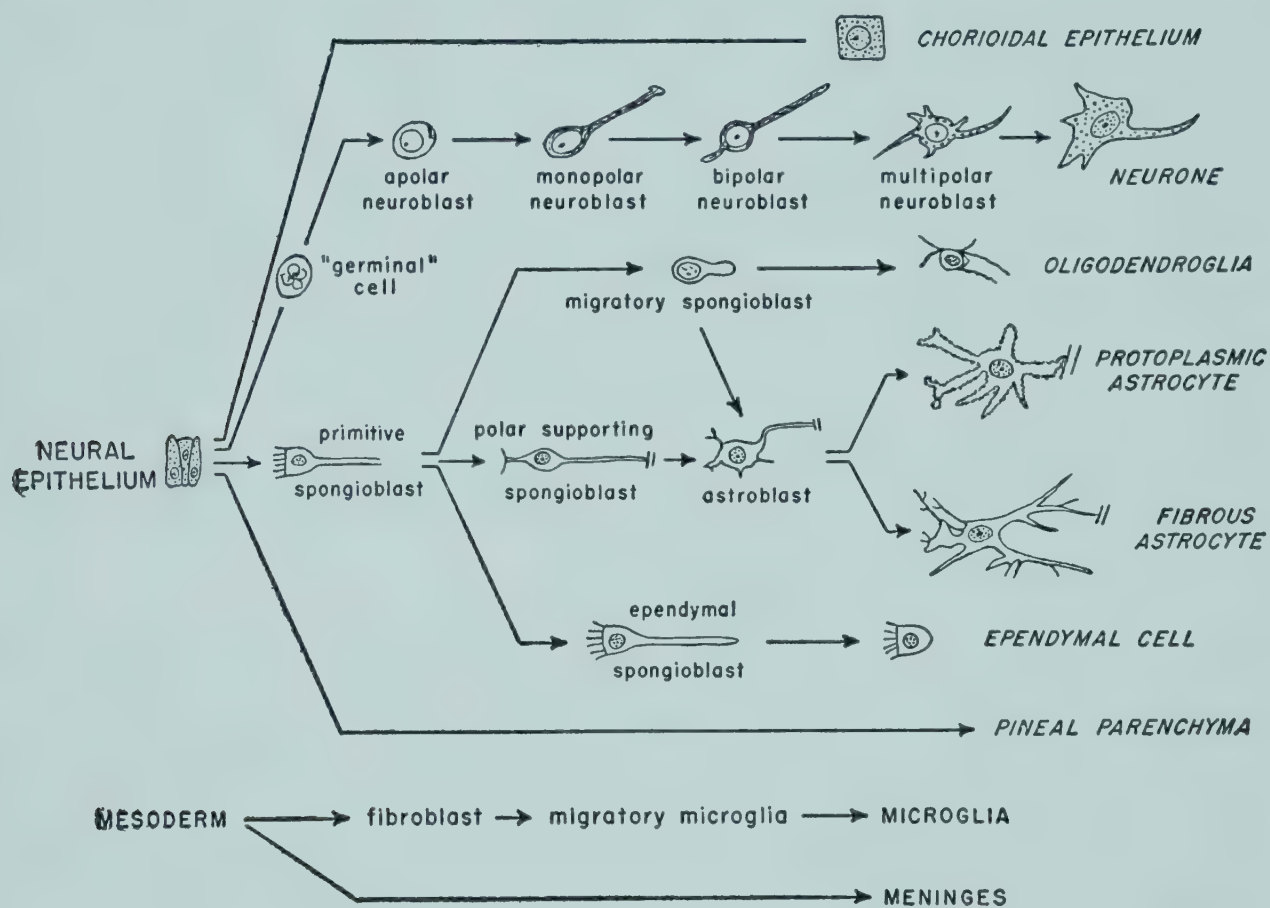


Fig. 139. A schema illustrating the probable origin and course of development of the nervous and nonnervous cells of the central nervous system. (Rearranged from various sources.)

by Falzacapa (1888). The microglia appear to be derived from the mesoderm. The sheath and capsular cells of the peripheral ganglia and nerve fibers are derived from the neural crest elements and possibly from the neural tube. The origin of the myelin is in doubt; some authors believe that it is produced by the sheath cells and others that it is produced by the axis cylinder. In either event, it is a product of ectodermal cells. A diagram showing the lineage of the various types of cells in the nervous system is presented in Fig. 139.

### Histogenesis of the Neuroglia and Ependyma

The early primitive spongioblast, with its cell body lying close to the neural canal, sends out a short process to the internal limiting membrane. This process often develops cilia that extend into the central canal; these



were seen by Klein (1880) in the 17-somite chick embryo. Each primitive spongioblast also forms a peripheral process which ends just beneath the external limiting membrane, or pia mater, in an enlarged footlike terminus. Many of these primitive spongioblasts retain this position and become the ependymal cells. Others, having established the supporting fibers, are referred to as supportive and polar spongioblasts. Still other spongioblasts migrate away from the central canal and lose their connection with it, thus becoming migratory spongioblasts.

The polar and supportive spongioblasts, and perhaps some of the migratory spongioblasts, develop footlike attachments to the invading blood vessels, thus becoming astroblasts and finally astrocytes. The oligodendroglia develop from the migratory spongioblasts slightly later than the astrocytes. They increase rapidly in number as myelinization occurs along the nerve fibers. Andrew and Ashworth (1945) have reported the presence of "oligodendroglia-like" cells which appear to have no processes, and they have termed them "adendroglia." Reddick (1951) has observed the differentiation of astrocytes and oligodendroglia from the neural epithelium *in vitro*, using smears of the postotic medulla of 2- to 7-day chick embryos. She stated that the early neuroglial cells have clear and distinct cytoplasmic boundaries, and that they do not assume the characteristic syncytial appearance until late in development.

As has been stated, the ependymal cells are derived from the primitive spongioblasts. The latter have long processes which reach from the cell body near the central canal to the external limiting membrane. During the later stages of development (beginning about on the fourteenth day in the chick), the peripheral processes atrophy and, in the adult, usually cannot be followed far from the cell body. In the region of the ventral median fissure and dorsal median sulcus of the spinal cord, however, these processes tend to remain for a long time.

### *Histogenesis of the Microglia*

The microglia do not appear until late, during the period when the meninges and blood vessels of the central nervous system are developing. According to those authors who believe microglia to be of mesodermal origin, there are two sources of these cells: (1) from tela chorioidea (the line of attachment of the chorioid plexuses), and (2) from the pial adventitia that surrounds the blood vessels, and from the pia itself. There are authors who, denying that the microglial cells are mesodermal in origin, state that they are of ependymal origin and thus ectodermal derivatives.

### *Sheath Cells and Capsular Cells*

The peripheral nerves have three sheaths of different morphological and functional significance: (1) the connective tissue endo- and perineurium;



(2) the neurilemma or sheath of Schwann; and (3) the myelin sheath. The first is composed of typical connective tissue elements and will not be discussed here. The myelin sheath will be discussed shortly. The sheath of Schwann, a thin envelope of cytoplasm with scattered nuclei, extends over each peripheral nerve fiber and is continuous with the capsular cell sheath of the ganglion cells. These sheath and capsular cells are derived from undifferentiated cells which migrate into the periphery in company with early neuroblasts. They come from the neural crest, via the dorsal root ganglia and cranial ganglia; and some, possibly, are derived from the neural tube proper.

In the early stages of development, these cells form what appears to be a syncytium within which pass nerve fibers and which also contains migrating neuroblasts. In the region of a developing ganglion, many of the undifferentiated cells invest the neuroblasts and differentiate into capsular cells. The remainder envelop the individual nerve fibers and differentiate into the cells of Schwann. The ovoid shape of the nuclei, arranged parallel to the course of the nerve fibers, distinguishes these cells from those of the adjacent mesenchyme.

Sheath cells, although forming not so complete an envelope, occur in the central nervous system. They are probably oligodendroglia.

### *The Myelin Sheath*

The degree of myelination of nerve fibers, both within and without the central nervous system, varies greatly. Some fibers are without (or have very little) myelin; others are slightly myelinated; and many are covered by a heavy sheath of neurokeratin material arranged in an irregular network, in the interstices of which fatty material, or myelin, is located. Myelination occurs selectively along the nerve fibers of certain tracts and nerves during the embryonic period and continues after hatching. The integrity of the myelin sheath in the normal animal is dependent upon the well-being of the neurone. If the neurone is damaged, the myelin sheath undergoes a fatty degeneration concurrently with the atrophy of the axis cylinder. When regeneration of a nerve fiber occurs, the myelin sheath is reestablished.

The origin of the myelin is not known. Some authors believe that it is produced by the sheath cells, although, as has already been stated, two different types of cells form the sheath, the cells of Schwann in the peripheral nervous system and the oligodendroglia in the central nervous system. Other authors believe that the axis cylinder itself elaborates the myelin. This would agree with the fact of differential myelination and with the facts of degeneration and regeneration. It is also possible that some interaction between the axis cylinder and its sheath cells induces the formation of the myelin sheath. According to observations made by Peterson



and Murray (1955) on chick embryo spinal ganglia of 4 to 15 days' incubation *in vitro*, myelin formation is possible only when a considerable protein reserve is present in the neurone soma; both nerve cell and Schwann cell are necessary to the elaboration of the myelin sheath.

No myelin sheaths are present on the sciatic nerves of the 9-day chick embryo. A few medullated fibers are first seen during the fifteenth day of incubation. It is interesting, however, that propagated action potentials can be recorded upon electrical stimulation of the unmyelinated nerve. The conduction rate is about 15 to 20 cm. per second on the tenth day and about 4 m. per second at hatching (Castillo and Vizoso, 1953).

### *Supporting Elements of the Spinal Cord*

Spongioblasts appear in the spinal cord of the chick embryo on the ninth day of incubation (Ramon, 1890a; Imhof, 1905). They appear first in the ventral horn, then in the white columns, and last in the dorsal horn (Ramon, 1890a). The presence of detached ependymal cells from the seventh to the fourteenth day of the chick's incubation suggested to Ramon (1890a) that these cells were primordial spongioblasts. By the ninth day, all the neural epithelium lining the central canal has assumed the character of a true ependymal layer (Hamburger, 1948).

Ramon (1890a) has described the neuroglial cells in the 9-day chick embryo (Fig. 115-E<sub>2</sub>). They vary in arrangement in different parts of the cord. In the region of the roof plate (except in the lumbosacral region), they form a parallel band between the central canal and the pia and give an indication of a dorsomedial septum. In the region of the ventral commissure (floor plate), those near the midline are vertically arranged, but those more lateral are displaced in an arc toward the periphery. Near the central canal, the cell bodies form the ependymal epithelium of the commissure, but peripherally the fibers of these cells are crossed by the fibers of the commissure and have many fine processes. The neuroglial cells in other regions of the cord are radially arranged, the cell bodies forming the ependymal epithelium of the central canal upon which are often seen fine cilia. The peripheral fibers of these cells branch and anastomose, and terminate in the pia mater.

### *Supporting Elements of the Autonomic Nervous System*

Jones (1938, 1939) and Brizzee (1949b) have discussed the formation of capsular, sheath, and other supporting cells of the peripheral autonomic nervous system. Both authors have concluded that these elements are formed by cells arising in the neural crest and in the neural tube. According to Brizzee (1949b), by 7 days of incubation, in the chick embryo, the majority of indifferent cells in the paravertebral trunk and in the aortic and prevertebral plexuses have differentiated into either supporting cells



or sympathoblasts. Most of the supporting cells have the appearance of oligodendroglia, and many neurilemma-like cells are arranged along the nerve fibers. At 11 days of incubation, four types of supporting cells were identified by Brizzee (1949b): (1) oligodendroglia-like cells of the Rio-Hortega Type 2; (2) cells with microglia-like nuclei, but with cytoplasm like the previous type of cell; (3) neurilemma-like cells; and (4) apolar cells which correspond to the adendroglia cells of Andrew and Ashworth (1945). The first indication of capsule formation was observed in one case on the fifteenth to eighteenth day of incubation, in another on the nineteenth to twentieth day, and in most cases not until shortly after hatching. In agreement with Pietra (1937) and Kuntz and Sulkin (1947), he concluded that capsule cells, as well as the other supporting elements of the autonomic nervous system, are of the oligodendroglia type, although some fibroblasts and microglia cells are present in the adult ganglia and plexuses.

### The Meninges

Surrounding the central nervous system are the meningeal membranes. The outer membrane is the dura mater or ectomeninx. In the cranial region it has two parts, its outer portion forming the periosteum on the inner surface of the cranial bones. Imbedded between the two layers of the dura in the cranial region are the great venous sinuses which drain the brain. In mammals, there are two membranes internal to the dura mater: the arachnoid and the pia mater. The inner meningeal membranes of birds, however, have been variously described.

Early workers (Cuvier, 1805; Owen, 1868) believed that the bird possesses all three meningeal membranes. Streeter (1904) stated that three distinct membranes are present in the meninges of the ostrich (*Struthio camelus*), and Hansen-Pruss (1923) described three in the chicken and the pigeon (*Columba livia*). Sterzi (1902) stated that there were only two membranes, but divided them in a peculiar fashion: (1) an outer nonvascular dura and (2) an inner secondary meninx subdivided into three portions: a thin outer endothelial layer, a middle vascular layer, and an inner layer adjacent to the neural tube. Farrar (1906) agreed that there were only two meninges in the bird, an outer dura and an inner pia-arachnoid. Harvey and Burr (1924) also described two meninges in the chick, the outer dura and the inner leptomeninx. Gelderen (1926) observed an ectomeninx (dura) and an endomeninx (pia-arachnoid) in the developing chick and duck (*Anas platyrhynchos*), and Cohen and Davies (1937) likewise distinguished two layers, (1) an outer dura mater and (2) an inner secondary meninx. The latter is trabeculated, but develops and functions as a single membrane.

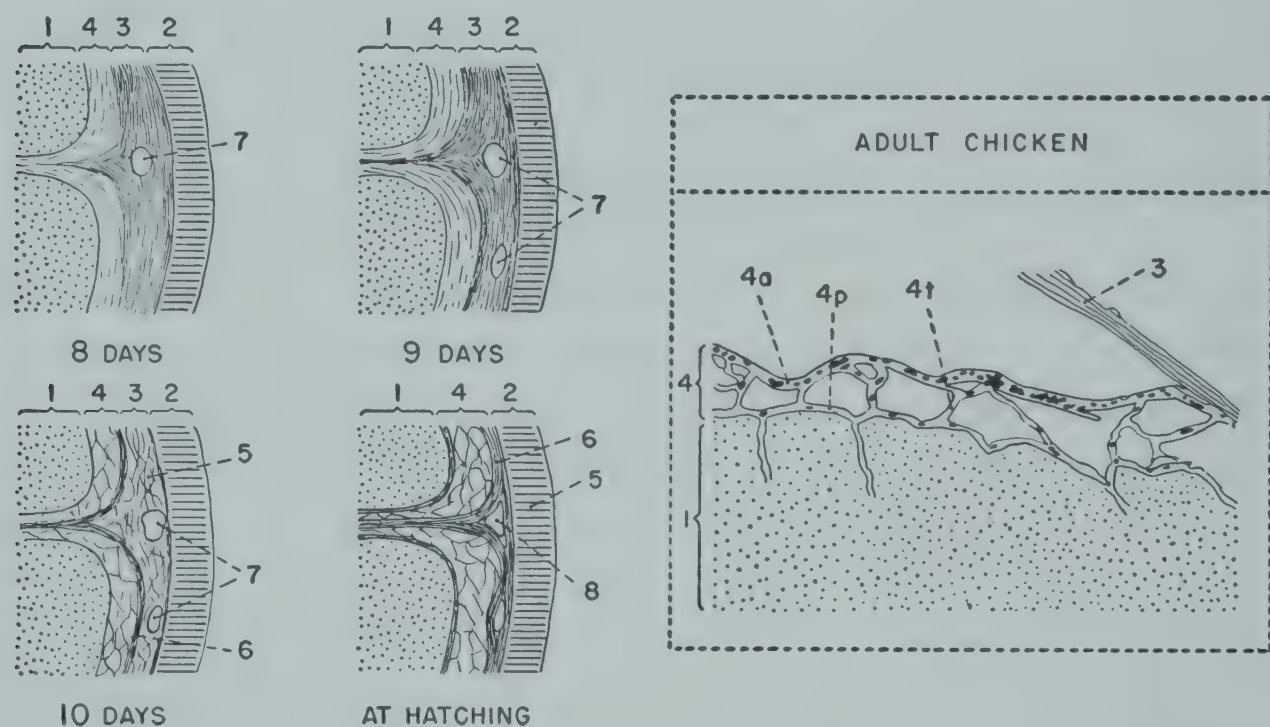
In birds, the existence of a functional subarachnoid space containing cerebrospinal fluid is controversial. Hansen-Pruss (1923), who identified



three meningeal layers. also stated that cerebrospinal fluid was present in the subarachnoid space. Cohen and Davies (1937), who described only an undifferentiated inner pia-arachnoid, could find no cerebrospinal fluid within the endomeninx.

### *Origin and Development of the Meninges*

Gelderen (1926) described a perineural mesenchyme about the brain of the chick at 46 to 72 hours (and up to the middle of the fourth day), with no trace of any cranial bone formation. The same condition exists



**Fig. 140.** The development of the meninges of the chick embryo. Four stages from the eighth day of incubation to hatching, inclusive, are shown schematically. (Redrawn with modifications after Gelderen, 1926.)

*Insert:* A diagrammatic representation of the meninges of the adult chicken's spinal cord, showing the dura mater and the meshwork of the the endomeninx which Hansen-Pruss (1923) termed the subarachnoid spaces. (After Hansen-Pruss, 1923.)

1, neural tissue; 2, cranial bones; 3, ectomeninx (dura mater); 4, endomeninx; 4a, arachnoid (Hansen-Pruss, 1923); 4p, pia mater (Hansen-Pruss, 1923); 4t, arachnoid trabeculae (Hansen-Pruss, 1923); 5, endocranial dura mater; 6, secondary dura mater; 7, ectomeningeal blood vessels; 8, peridural sinus.

in the duck (*Anas platyrhynchos*) until about 6 days of incubation. From the fourth to seventh days in the chick (and from 6 to 8 days in the duck, *Anas platyrhynchos*), the primitive bones of the cranium begin to form and to enclose the brain. The mesenchyme thus enclosed between the primordial cranium and the brain has been termed the primitive meninx, and in it lie the primitive meningeal veins. At about 8.25 days in the chick (8.5 to 9 days in the duck, *Anas platyrhynchos*), the primitive meninx has differentiated into an outer ectomeninx and an inner endomeninx (Fig. 140). This differentiation begins basally. By the eighth day in the chick (or the ninth day in the duck, *Anas platyrhynchos*), it has



reached the dorsal midline, and the falx anlage has appeared. By 10 days (in the chick), the endomeninx has thickened as a typical pia-arachnoid and has become firmly attached to the surface of the brain (cf. Fig. 140). The ectomeninx shows an inner secondary dural layer and an outer pericranial dural layer. The final adult meningeal pattern (cf. Fig. 140) is well established prior to the time of hatching. The development of the dural and other cranial blood vessels has been discussed in Chapter 8.

Harvey and Burr (1924) indicated that the dura and the leptomeninx do not both differentiate from the perineural mesenchyme, but that the leptomeninx is derived from the neural crest. There seems to be no evidence to support this assertion, although there may be some contribution of cells of ectodermal origin to the deep layers of the pia mater.

The question of whether or not the avian endomeninx may be subdivided into a pia and an arachnoid and the question of a subarachnoid space in birds require further study. Hansen-Pruss (1923) observed a subarachnoid meshwork in the endomeninx and differentiated both a pia and an arachnoid (cf. Fig. 140). That the endomeninx contains spaces is generally accepted (*Sterzi, 1902; Cohen and Davies, 1937*), but whether or not Hansen-Pruss (1923) was correct in terming these spaces subarachnoid spaces has not been established.

Cohen and Davies (1937) concluded that cerebrospinal fluid first escapes through the roof of the fourth ventricle after 7 days of incubation. The initial passage of fluid coincides with the functional establishment of the chorioid plexuses of the third and lateral ventricles, and the spread of fluid is enhanced by the functional development of the chorioid plexus of the fourth ventricle at the 8.5-day stage. According to these authors, the fluid passes into the subdural space rather than into the spaces of the endomeninx. Thus the endomeninx, although loosely trabeculated, should be considered not as a pia mater and an arachnoid, but as one single functional membrane.

### The Chorioid Plexuses

Chorioid plexuses are clearly recognizable in the lateral and third ventricles of the chick's brain before villi develop in the fourth ventricle. The first indication of the formation of chorioid villi is seen in the 6-day embryo as two or three slight folds of the thin roof of the diencephalon. Late in the seventh day, these folds are more pronounced and extend into the foramina of Monro. At the end of the seventh day, they branch to form incipient villi (cf. Fig. 94-I). During the eighth and ninth days, the folds continue to elongate and to become narrower and more branching. Each villus consists of mesenchyme containing capillaries and surrounded by epithelium. The chorioid plexus of the fourth ventricle begins its development at the 7.5-day stage as undulations of the ventrolateral wall of



the transverse fold crossing the roof of the myelencephalon. In the 8-day embryo, these folds have formed short, unbranched, incipient villi concentrated in two poorly defined groups, one at each side. Branching of the villi begins after 8.5 days' incubation and becomes fairly pronounced by the 9-day stage (*Cohen and Davies, 1937*).

By the chick's fifteenth day of incubation, the lateral ventricles have become mere slits, each containing a chorioid plexus which now exhibits very numerous branched villi. The villi at the base of the plexus are covered with two or three layers of epithelium, but the remainder possess only a single layer of columnar epithelium 18 to 20 microns high. The lateral branches contain many connective tissue nuclei. The chorioid plexus of the third ventricle is made up of elongated, often filamentous villi covered with columnar epithelial cells (height 20 microns) whose free surface bears cilia 7 to 8 microns high. The chorioid plexus of the fourth ventricle is also invested with ciliated epithelium, but is made up of short villi. By the twentieth day of incubation, the majority of villi in the chorioid plexuses of the lateral ventricles are also covered with ciliated epithelium, and the chorioid plexus of the fourth ventricle contains many more villi than on the fifteenth day (*Klosovskii and Kiseleva, 1935*).

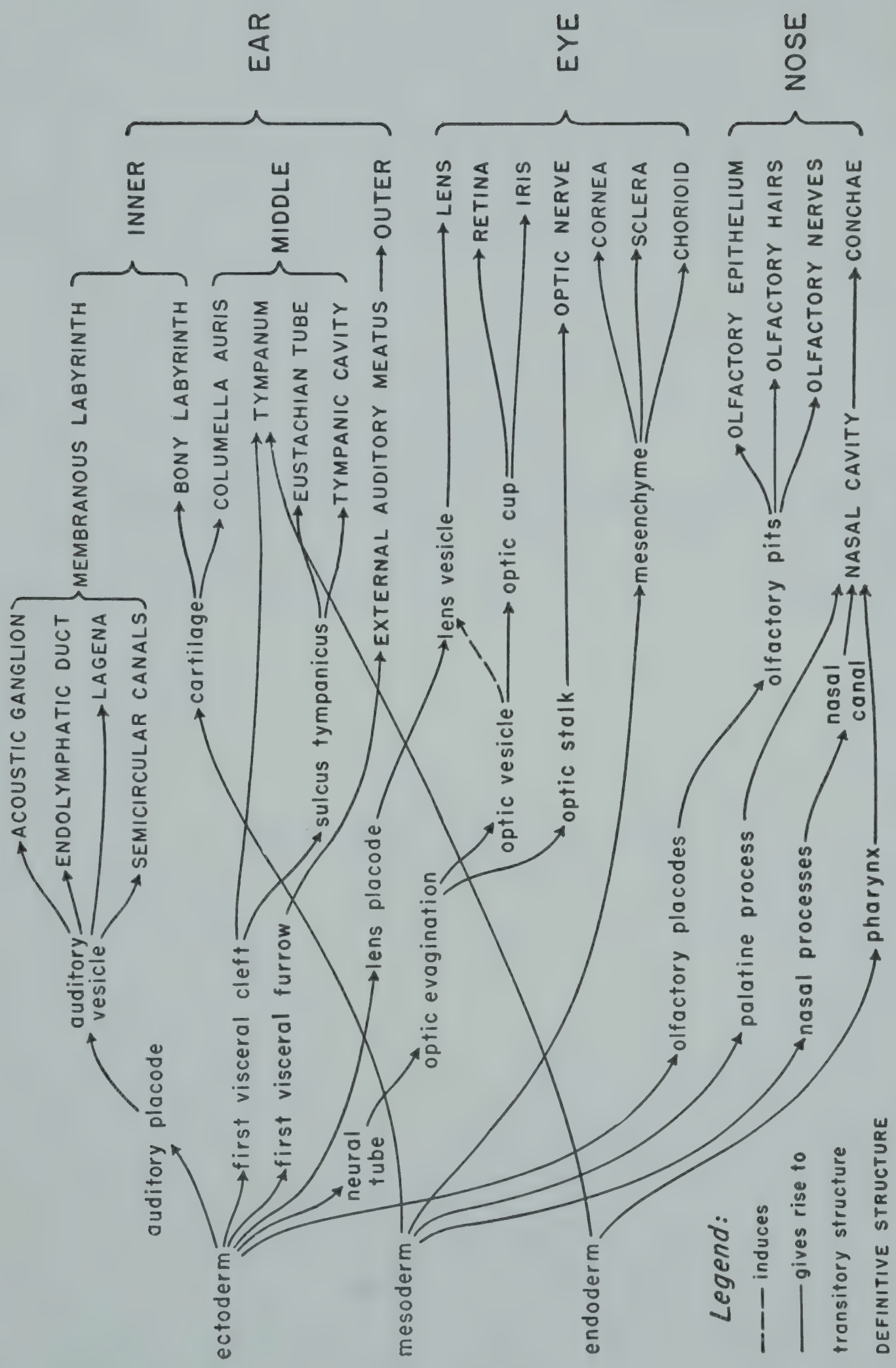


## CHAPTER FIVE

# The Organs of Special Sense

*These organs, keenly penetrating  
The outside world, give feeling, sense,  
Each creature wholly dominating  
By their direction and defense.*





DEVELOPMENT OF THE ORGANS OF SPECIAL SENSE IN THE AVIAN EMBRYO



# THE ORGANS OF SPECIAL SENSE

The ears, the eyes, and the nose are the organs of the three predominating senses. The development of these organs is closely related to that of portions of the nervous system. Each organ consists of a sensory region, in direct contact with the nervous system, and of accessory parts which serve to intermediate between the external environment and the sensory cells. The sensory tissue, like the nervous system, is derived from ectoderm; mesoderm contributes to the remainder of each organ to a varying extent. The formation of placodes or thickened areas of ectoderm is a feature common to the early development of all three sense organs.

## THE EAR

Although the bird's ear is similar to the mammalian organ, it exhibits a number of differences. The inner ear, or labyrinth, is the combined organ of equilibrium and hearing, supplied by the vestibular and cochlear nerves. The membranous labyrinth is a complex structure enclosed in a cavity of approximately the same shape (the perilymphatic cavity) and surrounded by the bony labyrinth. The membranous labyrinth is filled with fluid (endolymph). The central portion of the labyrinth consists of two intercommunicating sacs, the utricle and the saccule. Three hollow tubes, the semicircular canals, form arcs both originating and terminating in the utricle. Each canal is in a different plane: the superior canal, on the dorsal side of the utricle, parallels the sagittal plane; the lateral canal, on the lateral side, lies longitudinally in the horizontal plane; and the posterior canal, also on the lateral side, lies vertically in the transverse or frontal plane. A swelling, or ampulla, is present at one end of each canal. From the saccule, which is ventral to the utricle, the tubular endolymphatic duct extends upward and medially to terminate blindly in an enlargement, the saccus endolymphaticus, which lies close to the medulla. Another tubular structure, the lagena, curves in a slightly spiral course ventrally and medially from the saccule and ends in a small blind sac. The lagena corresponds to the cochlea of mammals.

The sensory portions of the inner ear consist of small areas of ciliated sensory epithelium in contact with nerve fibers. Such areas are found in



the ampullae of the semicircular canals, where they are known as the cristae acusticae, and in the utricle and the saccule, where they are termed maculae. In the lateral ampulla, the crista acustica consists of a transverse ridge of sensory epithelium, but in the other ampullae there is also a longitudinal ridge of epithelium-covered connective tissue, the septum cruciatum. The acoustic apparatus of the lagena is quite complex. The membranous lagena is supported in the perilymphatic space by a U-shaped cartilage whose arms (the external and internal cartilages) are continuous with each other distally. The external and internal cartilages divide the perilymphatic space into an anterolateral cavity, the scala vestibuli, and a posteromedial cavity, the scala tympani. The anterolateral wall of the membranous lagena is known as the tegmentum vasculosum and is probably secretory in function (*Amerlinck, 1923*). The sensory cells are confined to the papilla acustica basilaris, on the posteromedial wall of the membranous lagena, and to the papilla acustica lagenae in the terminal sac. The papilla acustica basilaris rests upon the membrana basilaris, and its internal surface is covered by the fibrous membrana tectoria. Sharply defined zones of tall columnar cells intervene between the tegmentum vasculosum and the papilla acustica basilaris. Within the distal enlargement of the lagena, the cavity contains otoliths, and the tectorial membrane is not present.

The middle ear, as in mammals, consists of the tympanic cavity bounded externally by the tympanic membrane or eardrum. The middle ear communicates with the pharyngeal cavity by means of the Eustachian tube, but the relationships between the pharynx and the tube are not exactly the same as in mammals. Instead of three auditory ossicles extending between the eardrum and the inner ear, birds possess only one, the columella auris, attached to the tympanic membrane by ligaments and muscle. Medially, the basal plate of the columella auris is in contact with the membranous fenestra oralis, which separates the tympanic cavity from the scala vestibuli. A similar membrane, the fenestra rotunda, separates the tympanic cavity from the scala tympani.

The outer ear consists of a relatively shallow external auditory meatus. There is no auricle.

The embryonic origin of the inner ear is entirely different from that of the other parts of the ear. The labyrinth, which contains all the sensory cells, is derived from ectoderm. The middle and outer ears, however, develop from mesodermal and endodermal tissue as well as from ectodermal.

### The Inner Ear

The development of the inner ear starts in the chick embryo of 7 or 8 somites as a very slight thickening of the ectoderm in a small area



on either side of the rhombencephalic region, approximately at the level of the anterior intestinal portal (*Poli, 1897*). A slight depression is detectable in this thickened ectoderm at the 9- to 10-somite stage. It is interesting to note that this appears to be the stage at which the presumptive ear ectoderm also loses the capacity for regeneration (*Levi-Montalcini, 1946a*) and acquires the capacity for self-differentiation (*Waddington, 1937b*). The induction of the auditory placode seems to be due to the combined action of neural tissue (the wall of the neural tube) and unidentified nonneural structures (*Waddington, 1937b*). The later differentiation of the labyrinth can occur in chorioallantoic grafts in the complete absence of nerve tissue (*Stcherbatov, 1938*).

The auditory ectoderm becomes visibly invaginated in the 11- to 13-somite chick (*Poli, 1897; Campenhout, 1937a*), lapwing, *Vanellus vanellus* (*Grosser and Tandler, 1909*), or zebra parakeet, *Melopsittacus undulatus*, embryo (*Abraham, 1901*). The early otic invagination is located directly anterior to the first somite (*Poli, 1897*). From the 15- to the 25-somite stage, the process of invagination produces a continually deepening pit with walls of stratified cylindrical epithelium, which is thickest at the bottom of the pit (*Röthig and Brugsch, 1902; Campenhout, 1937a*). Over the period extending from the 20- to the 30-somite stage, there is an apparent backward migration of the auditory pit from the level of the first visceral cleft to that of the second (*Poli, 1897*).

The transformation of the auditory pit into a vesicle is presaged in the 20-somite embryo by a slight narrowing of the external opening of the pit. After the 25-somite stage, closure proceeds rapidly and is completed at approximately the 30-somite stage (*Grosser and Tandler, 1909; Campenhout, 1937a*), at the time when cells start to bud off the medioventral surface of the vesicle in large numbers, to form the acoustic ganglion (see Chapter 4). The closed vesicle then gradually separates from the superficial ectoderm of the head, to which it is attached on its lateral surface. Eventually, the connection consists merely of a strand of cells (*Keibel, 1899; Röthig and Brugsch, 1902*), which finally disappears at some time after the 35-somite (*Campenhout, 1937a*) or 40-somite stage (*Grosser and Tandler, 1909; Fuchs, 1923*).

While the auditory pit is still open, it becomes ellipsoid in shape (*Fuchs, 1923*). By the time closure is effected, it has become pyriform or teardrop-shaped (*Röthig and Brugsch, 1902; Krause, 1906; Fuchs, 1923*), with its longest diameter directed dorsoventrally with reference to the straightened longitudinal axis of the embryo (Fig. 141-A<sub>1</sub>). The pointed dorsal extremity apparently represents the earliest anlage of the ductus endolymphaticus, which thus arises as an evagination of the dorsal (or dorsomedial) wall of the otocyst at a time when the lateral wall is still attached to the ectoderm (*Poli, 1897; Fuchs, 1923*). It should be mentioned



at this point, however, that the origin of the ductus endolymphaticus has been a matter of dispute. Röthig and Brugsch (1902) stated that the anlage of the duct does not appear until separation of the otocyst from the ectoderm is complete (although they showed the dorsal prolongation in an illustration of an earlier stage). Like Keibel (1899), these authors concluded that the duct is formed of cellular material that migrates to a medial position from the last part of the lateral wall of the auditory vesicle to separate from the ectoderm. According to Hasse (1867) and Krause (1901, 1906), the duct originates as a hollow tube connecting the otocyst with the surface epithelium, but neither Röthig and Brugsch (1902) nor Fuchs (1923) found a lumen in the cellular bridge between the otocyst and the ectoderm.

The pear-shaped otic vesicle exhibits a slight evagination on its anterior surface and another posteriorly (Fig. 141-A<sub>1</sub> and A<sub>2</sub>). These prominences are the earliest primordia of the superior and posterior semicircular canals, respectively (Fuchs, 1923).

After the separation of the vesicle from the ectoderm, there is an upward growth of the dorsal evagination, an extension of the anterior evagination on the lateral wall, and a slight outgrowth of the fundus of the vesicle to form the primordium of the lagena (Fig. 141-B). Shallow furrows separate these weak outpocketings on the surface of the still pyriform otocyst.

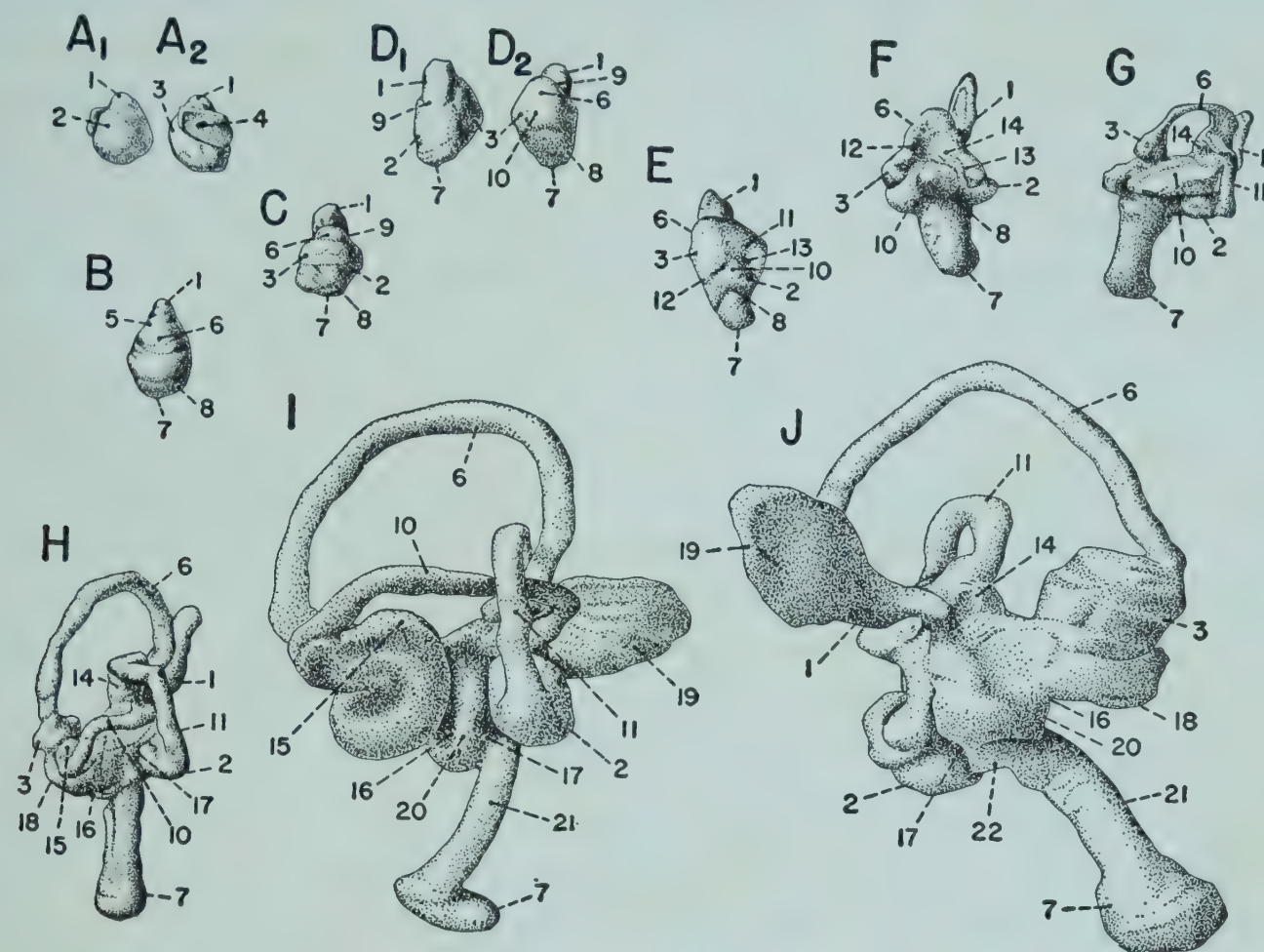
At approximately the 46-somite stage, the otic vesicle loses its teardrop shape because of an advance in the development of both the dorsal evagination and the now ridgelike primordium of the superior semicircular canal (Fig. 141-C). The enlargement of the latter structure has brought it to the dorsal surface and has resulted in the formation of a fairly deep furrow between it and the early endolymphatic duct, which has been shifted to the medial surface of the vesicle. The anlage of the posterior semicircular canal also has increased in size.

At the 48-somite stage, the endolymphatic duct is stalklike, and its base is demarcated by a furrow on both the medial and the lateral sides. The dorsal surface of the vesicle bulges more markedly than before. On the lateral surface another small evagination has appeared; this is the primordium of the lateral semicircular canal (Fig. 141-D<sub>1</sub> and D<sub>2</sub>). At the 51-somite stage, the distal extremity of the endolymphatic duct enlarges, initiating the development of the saccus endolymphaticus. At the same time, the early lagena begins to grow more rapidly.

At this or a slightly more advanced stage, the formation of two new furrows on the lateral surface of the otocyst (Fig. 141-E) starts the transformation of the evaginations into pouches, which will undergo a further transformation into semicircular canals. One of these furrows crosses the short diameter of the otocyst on its anterolateral aspect and



marks the boundary between the superior semicircular canal, or pouch, and the lateral pouch; the latter is separated from the lagena by the furrow formed previously. The second new furrow lies on the posterolateral surface and parallels the long diameter of the otocyst. It indicates the



**Fig. 141.** Successive stages in the development of the left labyrinth in the embryo of the European lapwing, *Vanellus vanellus*. (Redrawn with modifications after Fuchs, 1923.)

A<sub>1</sub> and A<sub>2</sub>, at the stage of 38 to 40 somites, medial and lateral views, respectively; B, lateral view at approximately the 43-somite stage; C, lateral view at approximately the 46-somite stage; D<sub>1</sub> and D<sub>2</sub>, at the 48-somite stage, medial and lateral views, respectively; E, lateral view at approximately the 51-somite stage; F, lateral view at a stage corresponding to that of 4 days 18 hours in the chick (*Keibel and Abraham, 1900*); G, lateral view at a stage corresponding to that of 5 days 11 hours in the chick; H, lateral view at a stage corresponding to that of 7 days in the chick; I, lateral view at a stage corresponding to that of 8 days in the chick; J, medial view at a stage corresponding to that of 10 days in the chick. All  $\times 20$ .

1, ductus endolymphaticus or its primordium; 2, ampulla of posterior semicircular canal (or its primordium); 3, ampulla of superior semicircular canal (or its primordium); 4, depression marking the site of attachment of the otocyst to the ectoderm; 5, furrow demarcating the primordial ductus endolymphaticus; 6, superior semicircular canal (or its primordium); 7, lagena (or its primordium); 8, furrow between lagena and lateral semicircular canal (or their primordia); 9, furrow between ductus endolymphaticus and primordial superior semicircular canal; 10, lateral semicircular canal (or its primordium); 11, posterior semicircular canal (or its primordium); 12, furrow demarcating primordial superior semicircular canal; 13, furrow demarcating primordial posterior semicircular canal; 14, superior sinus of utriculus (or its primordium); 15, ampulla of lateral semicircular canal; 16, 17, furrows demarcating saccus; 18, recessus utriculi; 19, saccus endolymphaticus; 20, saccus; 21, pars basilaris of lagena; 22, ductus reuniens of lagena.



anterior boundary of the posterior pouch, which at this time has the appearance of being a posterior extension of the superior pouch.

The further development of the labyrinth is best described with frequent reference to the accompanying illustrations. Inspection of Fig. 141-F (which represents a stage corresponding to that found in the 4.5-day chick embryo) shows that elongation of the endolymphatic duct and the lagena has occurred simultaneously with a deepening of the three furrows mentioned above. The shape of the superior and posterior semicircular canals is now clearly modelled; also, it can be perceived that the canals will be derived from the circumferential portions of the primordial pouches after collapse and perforation of the walls of the central portions. The swellings at the anterior end of the superior canal and the ventral end of the posterior canal are the respective early ampullae of these two canals. The lateral canal is as yet only a large round protuberance on the lateral surface of the vesicle. Another enlargement at the juncture of the smaller ends of the superior and posterior canals represents the early superior sinus of the utriculus. The posterior canal is beginning to swing around the long axis of the otocyst into its definitive position.

Figure 141-G shows the labyrinth of the lapwing (*Vanellus vanellus*) embryo at a stage of development equivalent to that found in the 5.5-day chick embryo. Here the superior and posterior canals are seen to have developed from their pouches, and their ampullae are clearly defined swellings. The lateral pouch is a flattened structure lying in the horizontal plane; the posterior canal arches around it dorsoventrally. The superior sinus of the utriculus is becoming more clearly defined at the point where the posterior ends of the superior canal and the lateral pouch meet the dorsal end of the posterior canal. The endolymphatic duct, originating from the medial wall of the labyrinth, has turned medially toward the brain. The lagena is thick and shows an enlargement at either end. The proximal enlargement is the primordium of the sacculus.

Figure 141-H shows the appearance of the lapwing (*Vanellus vanellus*) embryo's labyrinth after the perforation of the central portion of the lateral pouch to form the lateral semicircular canal. At this stage (found in the chick embryo of about 7 days' incubation), the definitive form of the utriculus is beginning to emerge. According to the description given by Fuchs (1923), this structure now has the shape of a T turned on its side. The short, normally horizontal arm of the T lies posteriorly and is directed dorsoventrally; its upper portion corresponds to the superior sinus, into which open the smaller ends of the semicircular canals, and its lower portion corresponds to the posterior sinus, to which the ampulla of the posterior canal is attached. The long arm of the T extends anteroposteriorly, its anterior extremity being represented by the anterior recess of the utriculus, with which the ampullae of the superior and lateral canals



communicate. The sacculus, above the proximal end of the now rather attenuated lagena, is somewhat enlarged and is beginning to be demarcated by two furrows, one running beneath the recessus utriculi and the other beneath the posterior ampulla.

Two further advanced stages in the development of the lapwing (*Vanellus vanellus*) embryo's labyrinth are shown in Fig. 141-I, which gives a lateral view, and in Fig. 141-J, which represents the medial aspect of the organ. These two stages are found in the chick embryo of about 8 and 10 days' incubation, respectively. In both illustrations, the sacculus is easily seen as a prominence bulging out above the root of the lagena.

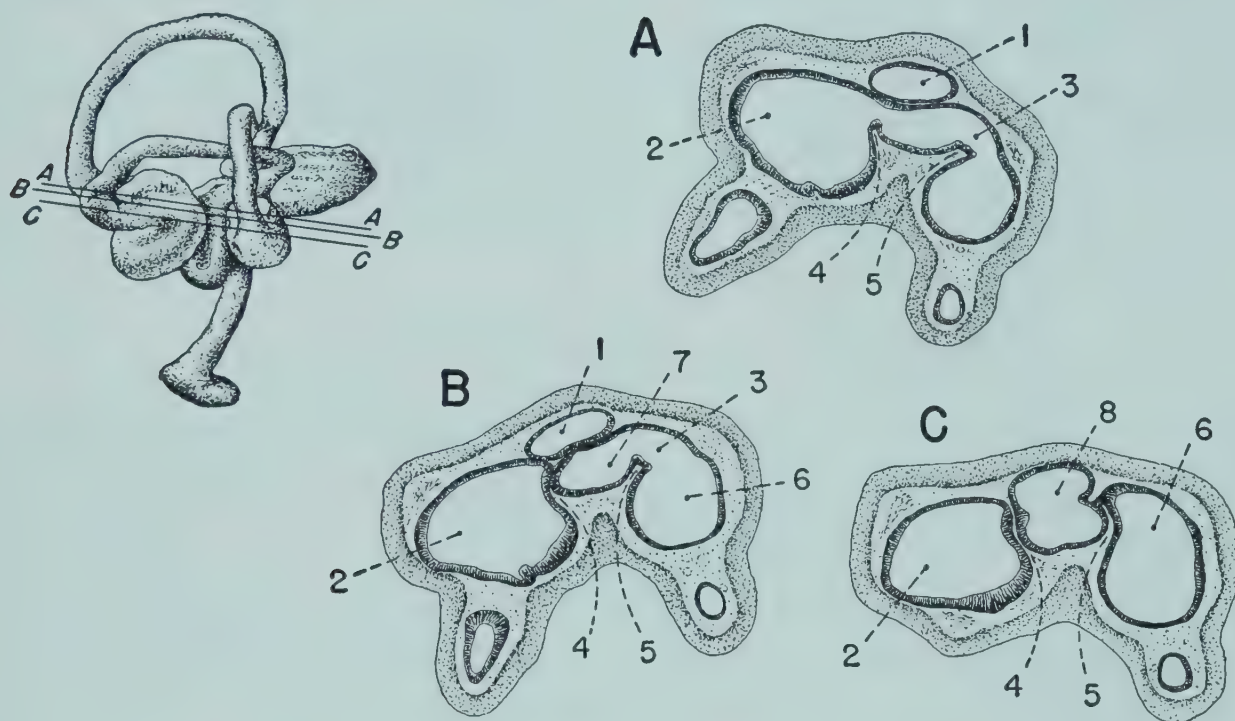


Fig. 142. Cross sections of the labyrinth of the European lapwing (*Vanellus vanellus*) embryo at a stage of development corresponding to that found in the chick (*Gallus gallus*) after 8 days' incubation. (Redrawn with modifications after Fuchs, 1923.)

A, B, and C, sections taken at levels A-A, B-B, and C-C, respectively, in the drawing at the left. All  $\times 15$ .

1, ductus endolymphaticus; 2, anterior recess of the utriculus; 3, posterior sinus of the utriculus; 4, 5, furrows demarcating the sacculus; 6, ampulla of the posterior semi-circular canal; 7, utriculosaccular foramen; 8, sacculus.

On the lateral surface, the two furrows previously mentioned separate the sacculus from the anterior recess and the posterior sinus of the utriculus; these furrows can be seen extending around on to the medial surface. The saccus endolymphaticus is broad and flat and lies close to the dorsal surface of the medulla, with its tip near the midline. The distal extremity of the lagena exhibits a hook-shaped enlargement. In the more advanced of the two stages shown here (cf. Fig. 141-J), the stalk of the lagena can be differentiated into a narrower proximal portion, the ductus reuniens, and a thicker distal portion, the pars basilaris. As is not easily seen in the illustration, the lagena bends first ventrolateralward on taking departure from the sacculus, then turns ventromedialward.



The internal relationships between the various components of the labyrinth are clarified in the three sections shown in Fig. 142. The most dorsal of these sections (Fig. 142-A) reveals that the external furrows delimiting the sacculus form internal ridges which divide the midportion of the utriculus from the broad anterior recess and the much narrower posterior sinus. The large, round ampulla of the posterior semicircular canal is seen to communicate with the posterior sinus. The endolymphatic duct and the lateral and posterior semicircular canals appear in cross section. A slightly more ventral section (Fig. 142-B) shows that the anterior recess of the utriculus has been isolated at this level by the medial extension of the more anterior of the two furrows in the lateral wall of the labyrinth. The midportion of the utriculus, at this level, forms a zone of communication between the utriculus and the sacculus. The third section (Fig. 142-C) is taken at the level where the endolymphatic duct takes origin from the medial surface of the sacculus; the plane of section no longer transects the posterior sinus.

### *The Sensory Epithelium of the Labyrinth*

Shortly after the otocyst loses its connection with the ectoderm, the wall of the vesicle—until now five or six cell layers thick—thins to three layers, except in the region bordering on the acoustic ganglion. By the time the endolymphatic duct has become stalklike (at approximately the 48-somite stage), the thickness of the lateral wall of the otocyst has decreased further to one or two layers, whereas the medial wall has become four- or five-layered. The lagena, in its early stages, is composed of epithelium four to six layers thick, thinning later to three layers on the lateral side (*Fuchs, 1923*).

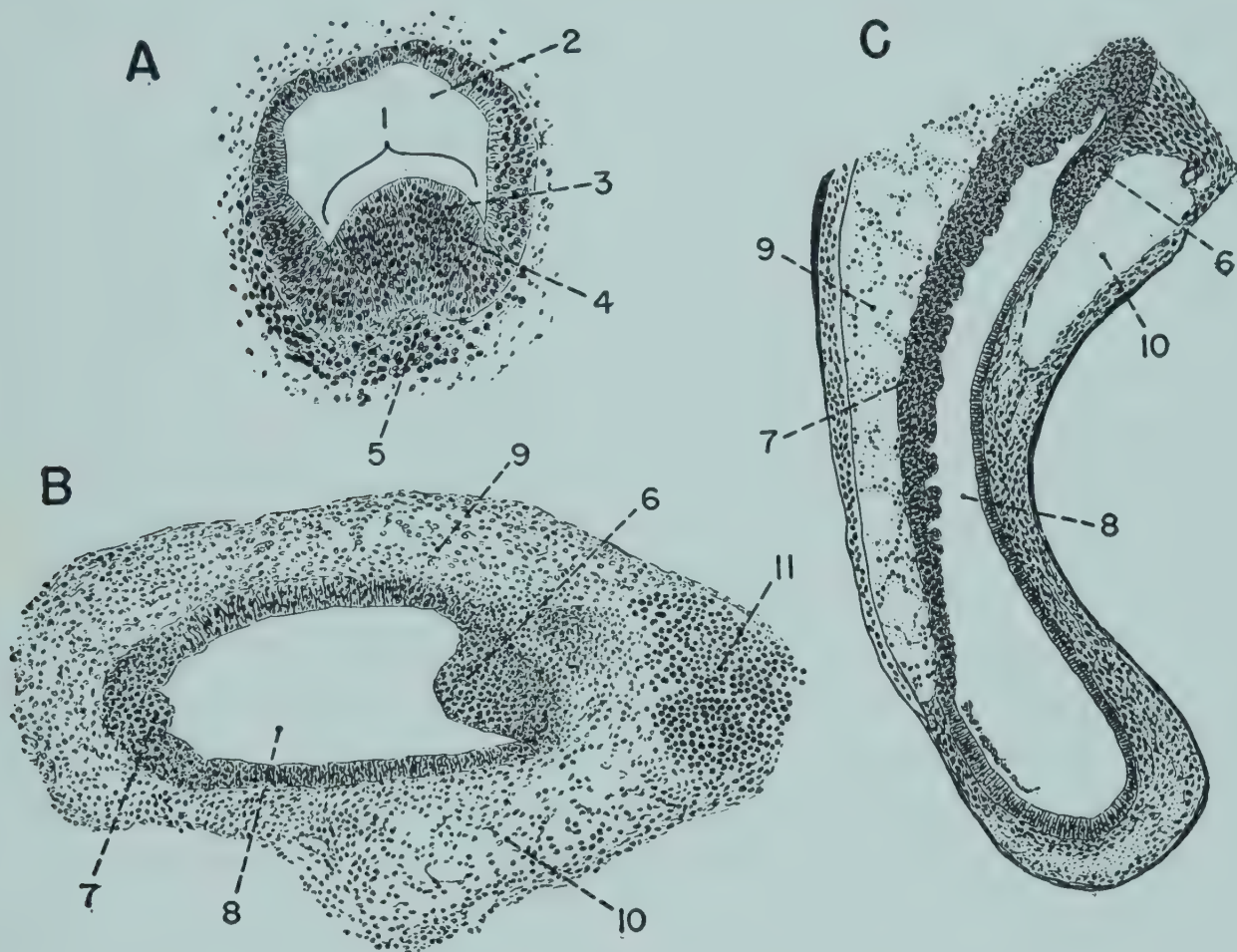
The localization of high epithelium in certain areas begins to be evident at a stage of development following the perforation of the superior and posterior pouches to form the corresponding semicircular canals. Thickened epithelium is now found in the ampullae of the superior and posterior canals and in the portion of the lateral pouch which will develop into the ampulla of the lateral canal (*Fuchs, 1923*).

Somewhat later, after the development of the lateral semicircular canal, a zone of epithelium five layers thick is found extending from the antero-lateral wall of the anterior recess of the utriculus into the lateral ampulla. A similar area is present on the medial wall of the posterior sinus (indicating the location of the future macula neglecta), on the medial wall of the sacculus, and, as before, in the superior and posterior ampullae. All these regions of thickened epithelium exhibit a clear, nucleus-free zone bordering the lumen of the labyrinth (*Fuchs, 1923*).

As the lagena lengthens, the thickened epithelium of its medial wall extends without interruption from the distal extremity throughout the pars



basilaris and is separated from the thickened epithelium of the medial wall of the sacculus by a zone of only slightly lower epithelium. The high epithelium of the sacculus is continuous with similar epithelium at the base of the endolymphatic duct. The macula neglecta, on the medial wall of the posterior sinus, is separated from the high epithelium of the posterior ampulla by a zone of lower cells. The sectioned appearance of the cristae acusticae at approximately this stage of development is indicated



**Fig. 143.** Various stages in the development of the inner ear in the chick embryo. (Redrawn with modifications A, after Amerlinck, 1930; B and C, after Amerlinck, 1923.)

A, section of an ampulla of a semicircular canal from an 8.5-day embryo ( $\times 160$ ); B, obliquely transverse section of the lagena of a 9-day embryo ( $\times 80$ ); C, longitudinal section through the lagena of a 10-day embryo. ( $\times 45$ ).

1, crista acustica; 2, lumen of ampulla; 3, nucleus of sensory cell; 4, nucleus of supporting cell; 5, connective tissue; 6, papilla acustica basilaris; 7, tegmentum vasculosum; 8, lumen of lagena; 9, region of future scala vestibuli; 10, region of future scala tympani; 11, ganglion.

in Fig. 143-A. As reference to Fig. 142-A, B, and C reveals, thickened epithelium is found also on the posterolateral surface of the anterior recess of the utriculus at this time, but its presence here is transitory (*Fuchs*, 1923).

At a late stage (cf. Fig. 141-I), the sensory epithelium of the primordial macula utriculi (on the floor and the anterior wall of the anterior recess) is still connected with that of the lateral ampulla. The macula neglecta of the posterior sinus is now clearly separated from the macula sacculi of



the medial wall of the sacculus and from the sensory epithelium of the posterior ampulla. Also, the low epithelium of the pars reuniens now intervenes between the macula sacculi and the early papilla acustica basilaris, and another zone of low epithelium separates the latter from the papilla acustica lagenae. An obliquely transverse section through the lagena of the 9-day chick embryo is shown in Fig. 143-B and a longitudinal section of the 10-day embryo's lagena is reproduced in Fig. 143-C.

The sensory tissue of the ear consists of special ciliated cells interspersed with supporting cells. In the chick embryo, the sensory cells begin to differentiate early in the second week of development.

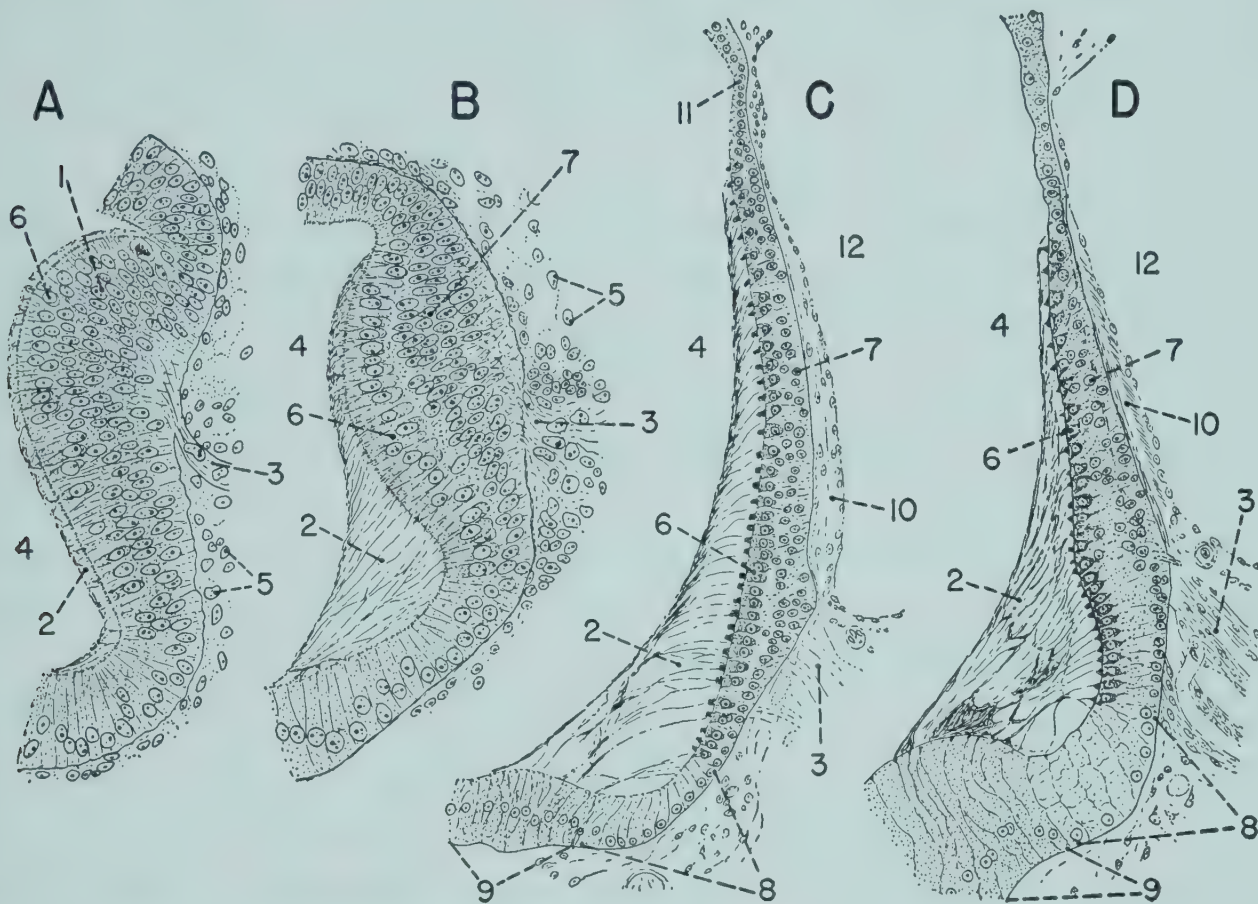
In the utricle, the most superficial cells of the primordial macula utriculi exhibit cilia after about 8.5 days of incubation. The underlying cells, which will differentiate as supporting cells, are alveolar and are traversed by nerve fibers. A day later, the sensory cells are numerous, their cilia are well developed, and a third type of cell, conical in shape, has appeared near the basement membrane. In the 18-day chick embryo, the pear-shaped, ciliated sensory cells are found between the summits of the supporting cells, whose nuclei are basal; and the conical cells, with elongated nuclei, line the basement membrane. In the nonsensory epithelium of the utricle, granular, darkly staining, flask-shaped cells are now visible. They appear to be secretory in nature and are abundant between the macula utriculi and the macula neglecta. The roof of the utricle, which was formed of cuboid to prismatic epithelium at 8.5 days, is composed of flat cells at 18 days of incubation (*Amerlinck, 1930*).

The development of the cristae acusticae parallels that of the macula utriculi except for the fact that no conical basal cells appear. The differentiation of the sensory and supporting cells of the cristae acusticae is practically complete in the chick embryo after 13 days' incubation, and secretory cells start to appear in the ampullae at this time (*Amerlinck, 1930*).

In the pars basilaris of the lagena, the differentiation of sensory cells is preceded by the appearance of the primordial membrana tectoria, or membrane of Corti, which can be seen in the lumen of the 7-day chick embryo's lagena (*Hasse, 1867; Held, 1909a*). The membrane is very delicate and extends across the posteromedial wall of the lagena, above the thickened epithelium from which the papilla acustica basilaris will develop. Numerous short, fine fibers connect the membrane with the epithelium. After 8 days of incubation, the membrane has started to grow on to the anteromedial wall of the lagena. A few sensory cells have differentiated in the uppermost layer of the epithelium and can be recognized chiefly by the cilia projecting from them into the endolymph in the lumen of the lagena (Fig. 144-A). In the 9-day embryo (Fig. 144-B), the tall ciliated cells form a superficial row, beneath which there are several rows



of elongated supporting cells with nuclei aligned perpendicular to the surface (*Held, 1909a; Amerlinck, 1930*). The lumen of the lagena is now wider, its anterior wall is longer, and the membrane of Corti stretches across the angle between the anteromedial and posteromedial walls, above the so-called cells of Deiters and the hyaline cells of the sulcus spiralis internus. By the tenth incubation day, the sensory cells start to become pointed basally, and the upper portions of the supporting cells penetrate between the sensory cells.



**Fig. 144.** The development of the organ of Corti in the ear of the chick embryo. (Redrawn after *Held, 1909a*.)

A, 8 days ( $\times 160$ ); B, 9 days ( $\times 160$ ); C, 16 days of incubation ( $\times 80$ ); D, a comparable section from the ear of an adult chicken ( $\times 80$ ).

1, undifferentiated sensory epithelial cells; 2, fibers of tectorial membrane; 3, cochlear nerve; 4, lumen of lagena; 5, cells in region of future scala tympani; 6, sensory cell of papilla acustica basilaris; 7, supporting cell; 8, hyaline cells; 9, cells of Deiters; 10, basilar membrane; 11, sulcus spiralis externus; 12, scala tympani.

The initial development of the basilar membrane is apparent on the eleventh day when this structure consists of two layers. Directly beneath the epithelium there is a very thin, noncellular layer. External to this there is a layer of fine parallel fibers extending from the internal to the external cartilage. The fibers are interspersed with oval nuclei, some of which lie perpendicular to the fibers. A few fusiform cells lie against the fibrous layer, in the perilymphatic space. The basilar membrane apparently arises from connective tissue cells which do not participate (by liquefaction) in the formation of the perilymphatic space (*Hasse, 1867; Amerlinck, 1930*).



After 13 to 15 days of incubation, the cilia of the sensory cells of the papilla acustica basilaris are grouped in tufts. The cilia are shorter, thicker, and less numerous than those in other regions of the ear (*Amerlinck, 1930*). By the sixteenth incubation day (Fig. 144-C), the lagena is considerably larger, and the sensory epithelium no longer bulges prominently into the lumen. The tectorial membrane has grown somewhat heavier superficially, but its deeper fibers are still fine and rather widely spaced. In the adult ear (Fig. 144-D), these fibers penetrate into the sensory epithelium as far as the free surfaces of the supporting cells, or perhaps farther. The end plates of the cochlear nerve fibers are in contact with the bases of the ciliated cells, or possibly fused with them (*Held, 1909a*).

The cells of Deiters, the hyaline cells, and the cells of the sulcus spiralis externus (which intervenes between the papilla acustica basilaris and the tegmentum vasculosum) are all represented in the 8-day chick embryo by cylindrical epithelium (cf. Fig. 144-A). Their subsequent development (cf. Fig. 144-B and C) is marked chiefly by a diminution in the height of the cells of the sulcus spiralis externus, by an increasing clarity of the cytoplasm of the hyaline cells, and by the migration of the nuclei to a basal position in the Deiters and hyaline cells and to a superficial position in the cells of the sulcus spiralis externus (*Amerlinck, 1923*).

The differentiation of the tegmentum vasculosum, which constitutes the posterolateral wall of the membranous lagena (cf. Fig. 143-C), begins in the chick embryo after 8 days of incubation (*Amerlinck, 1923*). At this time, the cells are starting to form groups separated by small grooves. Darkly staining elongated cells containing many mitochondria can be distinguished among the predominating large clear cells. About a day later, a thin layer of connective tissue appears basally, formed at the expense of neighboring cells that do not take part in the development of the perilymph. As incubation continues, this connective tissue provides axial support for many buds of epithelial cells that grow out, as villus-like projecting folds, into the lumen of the lagena. By the fifteenth day, the folds are widely spaced. On the eighteenth day, the darkly staining cells are found only in the folds, where they are very compressed, and are not present in the membrane lining the spaces between the folds (*Amerlinck, 1923*).

The papilla acustica lagena presumably develops in much the same manner as the papilla acustica basilaris. The development of the otoliths which overlie it is initiated by the appearance of a fine, transparent, gelatinous film, into which small spindle-shaped bodies are deposited. The latter can be seen in the 8-day chick embryo (*Hasse, 1867*).

Fell (*1928a*) observed that the otocyst of the 3-day chick embryo con-



tinues its histological differentiation after being explanted to clotted plasma and embryo extract. After 14 days *in vitro*, the structure may correspond topographically to that of a normal 17-day ear. Practically no growth occurs, however.

### *The Perilymphatic Space and the Bony Labyrinth*

The membranous labyrinth is surrounded by a mass of undifferentiated embryonic connective tissue until the second week of the chick embryo's incubation period. During this week, some of the cells in the mass differentiate *in situ*, forming the cartilage that supports the membranous labyrinth in the perilymphatic space (*Hasse, 1867*). The cells occupying the region of the future perilymphatic space are destined to undergo liquefaction. The beginning of this process is visible during the ninth day of incubation (cf. Fig. 143-B), when the cells are elongating and spreading apart; in some places, especially around the utricle and in the scala tympani, they are starting to liquefy. Liquefaction is very advanced on the thirteenth day but is not complete until the eighteenth day (*Amerlinck, 1930*).

On the ninth or tenth day of development, the bony labyrinth consists of embryonic cartilage cells. Ossification begins after 11 days (*Arai, 1927*) to 13 days of incubation (*Amerlinck, 1930*) and is marked after 15 days. It proceeds very slowly from the region of the utricle and sacculus outward (*Arai, 1927*). According to *Amerlinck (1930)*, ossification is complete on the eighteenth day, but *Arai (1927)* stated that the bony labyrinth does not entirely surround the membranous labyrinth at hatching time and that the regions around the fenestra ovalis and the basal plate of the columella auris are still cartilaginous.

### **The Middle and Outer Ears**

The structures of the middle and outer ears are derived from the region of the first visceral furrow. The earliest stage in their development is recognizable in the chick at the end of the third incubation day, when a small perforation is found at the dorsal end of this visceral furrow (*Kastschenko, 1887*). The perforation opens into the first visceral pouch (a dorsolaterally directed extension of the fore-gut) and thus forms a communication between the external surface and the pharyngeal cavity. The communicating passage has been termed the sulcus tubotympanicus (*Moldenhauer, 1877*); later it becomes the Eustachian tube. A horizontal section through the dorsal opening in the first visceral furrow is shown in Fig. 145-A. A somewhat more ventral section (Fig. 145-B) no longer transects the opening but shows that the sulcus tubotympanicus (or first visceral pouch) is continuous with the pharyngeal cavity. At this



level, mesoderm is present between the external ectoderm and the endoderm of the first visceral pouch. A lobular enlargement can be seen projecting into the pharyngeal cavity from the anterolateral wall of the latter, at the level of the mandibular arch; this is the colliculus palatopharyngeus, or palatine process of the maxillary process (*Moldenhauer, 1877*). A cross section (Fig. 146-A) through the mandibular arch, slightly cranial to the first visceral furrow, shows that the sulcus tubotympanicus is found at this level, also, and that it bounds the colliculus palatopharyngeus dorsally. Ventral to the colliculus palatopharyngeus there is a second (but ventrolaterally directed) extension of the fore-gut, the sulcus lingualis. Together, the sulcus tubotympanicus and the sulcus lingualis give the cross section of this region of the fore-gut the shape of a flattened X.

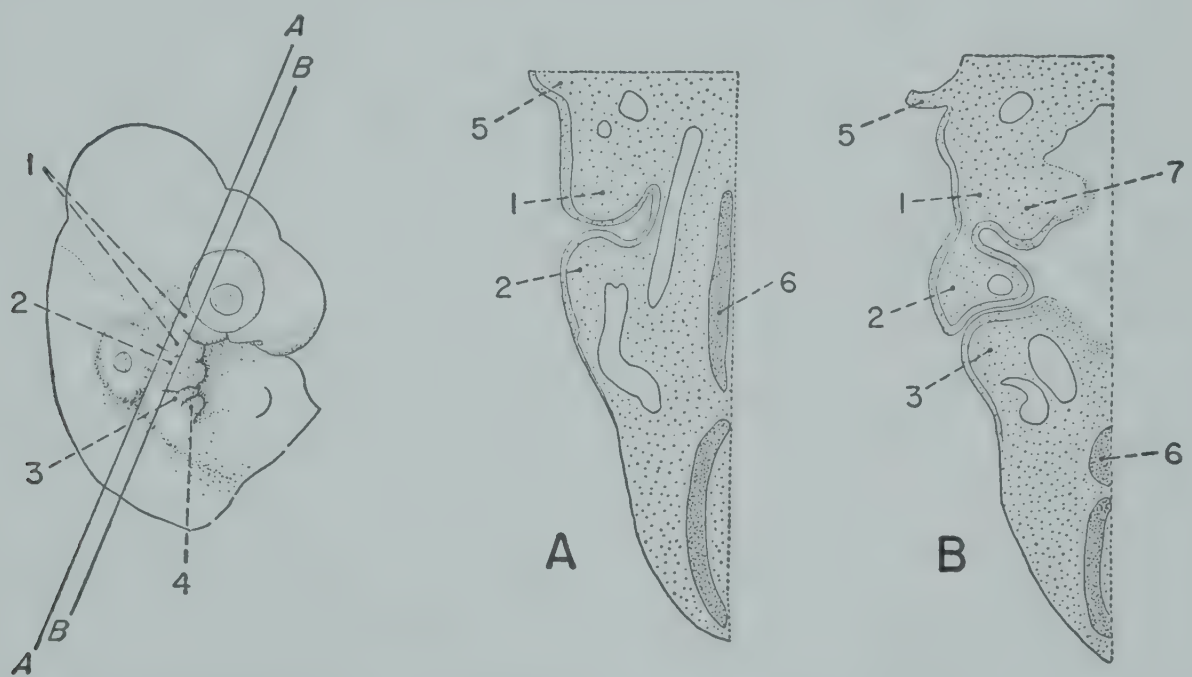


Fig. 145. Diagrammatic representations of horizontal sections through the pharyngeal region of the 4-day chick embryo. (Redrawn with modifications after *Moldenhauer, 1877*.)

A, a section through the dorsal end of the first visceral cleft ( $\times 20$ ); B, a section at a more ventral level ( $\times 20$ ). The approximate levels of sections A and B are indicated, respectively, by the lines A-A and B-B on the drawing at the left ( $\times 7$ ).

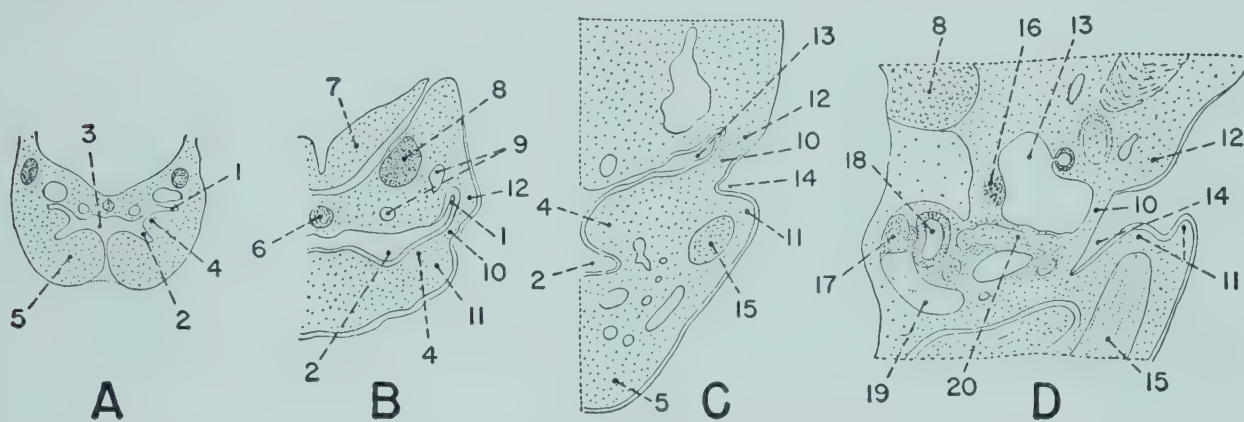
1, 2, 3, 4, first, second, third, and fourth visceral arches; 5, eye; 6, notochord; 7, colliculus palatopharyngeus.

By the sixth day of incubation, a depression of limited size has developed along the course of the first visceral furrow at a point not far ventral to its dorsal extremity. The ventral end of this deeper portion of the furrow has swung slightly caudad, so that the indentation runs somewhat diagonally to the remainder of the furrow. Four rounded protuberances or colliculi (*Moldenhauer, 1877*)—two on the mandibular process and two on the hyoid arch—surround the slitlike depression, which is clearly the primitive external auditory meatus. A cross section (Fig. 146-B) taken through the posterior part of the mandibular arch of the 6-day



chick embryo shows that the meatus is located directly external to a dorsal extension of the sulcus tubotympanicus, from which the meatus is separated by the anlage of the tympanic membrane. The posterior and anterior colliculi of the mandibular arch flank the meatus dorsally and ventrally. A general lateral growth of the region has brought about a flattening of such structures as the colliculus palatopharyngeus, the colliculus lingualis, and the sulcus lingualis.

By the ninth day of incubation, the distal portion of the sulcus tubotympanicus has enlarged to form the primordium of the tympanic cavity, and its proximal portion has become narrow and tubelike (Fig. 146-C). The auditory meatus has deepened considerably.



**Fig. 146.** Various stages in the development of the middle and the outer ear in the chick embryo, as seen in cross sections. (Redrawn with modifications after Moldenhauer, 1877.)

A, a section through the mandibular arch at 4 days; B, a section through the first pharyngeal furrow at 6 days (right side); C, a section through the early auditory meatus and tubotympanic cavity at 9 days (right side); D, a section through the external ear at 12 days (right side). All  $\times 11$ .

1, sulcus tubotympanicus; 2, sulcus lingualis; 3, oropharyngeal cavity; 4, colliculus palatopharyngeus; 5, mandibular process or mandible; 6, notochord; 7, medulla oblongata; 8, Gasserian ganglion; 9, branchial artery; 10, tympanic membrane; 11, anterior colliculus; 12, posterior colliculus; 13, tympanic cavity; 14, external and auditory meatus; 15, masseter muscle; 16, tympanic ganglion; 17, acoustic ganglion; 18, lagena; 19, perilymphatic canal; 20, columella auris.

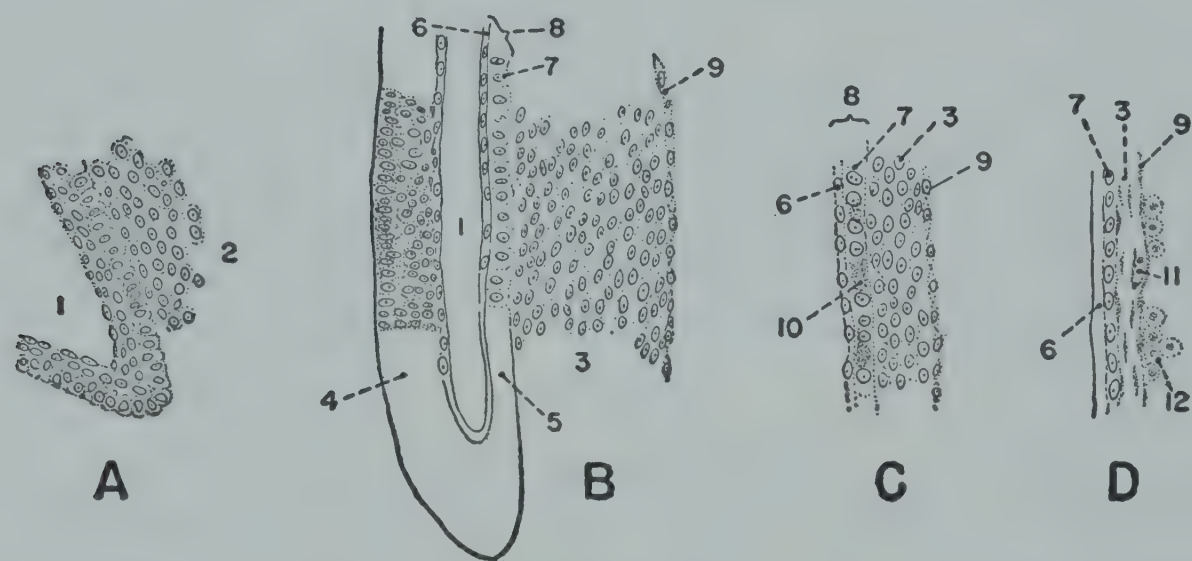
There is a great advance in the development of the outer and middle ears between the ninth and the twelfth day, as may be seen by comparing Fig. 146-C with Fig. 146-D (in which the Eustachian tube is not shown). The auditory meatus has now assumed much of the character of a passage. The tympanic cavity shows a great increase in size, which Moldenhauer (1877) attributed to resorption of tissue. The cartilaginous primordium of the columella auris (see Chapter 12) can be seen stretching between the lagena and the tympanum. There is a sharp transition from the walls of the auditory meatus to the tympanum, which is much thinner than at any previous time. The thickness of the chick's eardrum at various



stages of development, as determined by Moldenhauer (1877), is given herewith.

Age of Chick Embryo (incubation days)	Thickness of Tympanum (mm.)
6	0.11
9	0.14
12	0.072
(Adult)	(0.012)

Histological details at two stages in the development of the eardrum and the epithelium of the external auditory meatus are shown in Fig. 147. The section reproduced in Fig. 147-A is taken through the deepest part



*Fig. 147. Histological changes in the tympanic membrane and in the epithelium of the external auditory meatus of the chick during the course of embryonic development. (Redrawn after Moldenhauer, 1877.)*

A, a section through the bottom of the external auditory meatus of the 9-day embryo; B, a section through the same region in the 12-day embryo; C, a section of the tympanic membrane of the 14-day embryo; D, a section of the tympanic membrane of the adult chicken. All  $\times 180$ .

1, external auditory meatus; 2, mesoderm; 3, mesoderm of tympanic membrane; 4, epithelium of external auditory meatus; 5, outer epithelium of tympanic membrane; 6, stratum corneum; 7, stratum mucosum; 8, tissue of ectodermal origin; 9, tissue of endodermal origin; 10, pigment; 11, blood vessel; 12, blood corpuscle.

of the auditory meatus of the 9-day chick embryo. The ectoderm of the external canal is continuous with the ectoderm of the primitive tympanic membrane and is composed throughout of an inner layer of cuboidal cells, an outer layer of flattened cells, and an intermediate layer of round cells. The figure shows only a portion of the mesodermal layer (composed of round cells) and none of the endodermal layer of the eardrum. Figure 147-B represents a section through the same region of the 12-day embryo. In the epithelium of the auditory meatus, three or four layers of round cells are now interposed between the inner layer of pyramidal cells and the outer layer of flat cells lining the canal. The ectodermal elements



on the external surface of the tympanic membrane consist of an inner layer of cuboidal cells (the future stratum mucosum) and an outer layer of flat cells (the future stratum corneum). The latter cells are continuous with the layer of flat cells lining the auditory meatus. The endodermal elements, which face on the tympanic cavity, are represented by a single layer of flat cells. The major portion of the membrane is composed of cells of mesodermal origin, now somewhat flatter than before. Ten or twelve layers of these cells are found between the inner and outer surfaces. This intermediate mesodermal portion, however, soon starts to grow thinner. In the 14-day chick embryo (Fig. 147-C), it is composed of only four layers of still more distinctly flattened cells and is clearly the primordium of the basement membrane of the adult tympanum (Fig. 147-D).

### The Paratympanic Organ

A small vesicle, the paratympanic organ, is found dorsal and medial to the tympanic cavity of many birds (*Vitali, 1914*). Although its exact function is not known, it is obviously a sense organ, for it contains sensory epithelium and is innervated by nerve fibers from the geniculate ganglion. Studies of its embryonic development in the sparrow, *Passer domesticus* (*Vitali, 1911, 1912*), and the chick (*Benjamins, 1925; Yntema, 1944*) indicate that it arises from an ectodermal thickening at the dorsal edge of the second visceral cleft and that its origin is intimately related to that of the geniculate ganglion. The organ apparently migrates medially to attain its definitive position. After the seventh day of the chick's incubation period, a small "peripheral ganglion" (*Benjamins, 1925*) forms between the geniculate ganglion and the paratympanic organ, which has developed high sensory epithelium by this time. In the chick, the innervation of the organ begins on the eighth day of incubation.

## THE EYE

The bird's eye conforms to the general vertebrate type but contains various features which account for the extraordinary acuteness of vision characteristic of most avian species. The wall of the more or less spherical eyeball is composed of several concentric layers. Except over the anterior portion of the eye, these are, from the exterior inward, as follows: the fibrous sclerotic coat; the vascular, pigmented chorioid coat; the pigmented layer of the retina; and the sensory layer of the retina. Anteriorly, the sclerotic coat is continuous with the transparent cornea; the chorioid coat is continuous with the more external, muscular portion of the ciliary processes and the iris; and the retinal layers are continuous with the thin tissue lining the internal surface of the ciliary processes and the iris. The ciliary processes and the iris are discontinuous anteriorly. The circular



opening in the iris is the pupil, which is made to vary in size by the contraction of the iris muscles. Directly behind the iris, and obliquely behind the ciliary processes, lies the lens, formed of laminated fibers and somewhat flatter than spherical in shape. Between the lens and the cornea is the small, liquid-filled anterior chamber of the eye. Behind the lens is the large posterior chamber, filled with the gelatinous vitreous humor.

The most striking difference between the avian and the mammalian eye is the absence of retinal blood vessels and the presence, instead, of a special vascular structure, the pecten, projecting into the vitreous humor. Another difference is the fact that there are two or even three foveae (or regions of most acute vision) in the retina of certain avian species, notably birds of prey; in some birds, there is also a bandlike central area of distinct vision (Wood, 1917, *pp.* 19–22, 59–61). The ciliary and iris muscles are striated instead of smooth, and it is possible that accommodation is accomplished otherwise than in the mammalian eye. The sclerotic coat is reinforced in its anterior portion by bony plates. Most of these major differences represent adaptations for vision during flight and, directly or indirectly, contribute to the superiority of birds' eyesight over that of mammals.

### General Relationships in the Developing Eye

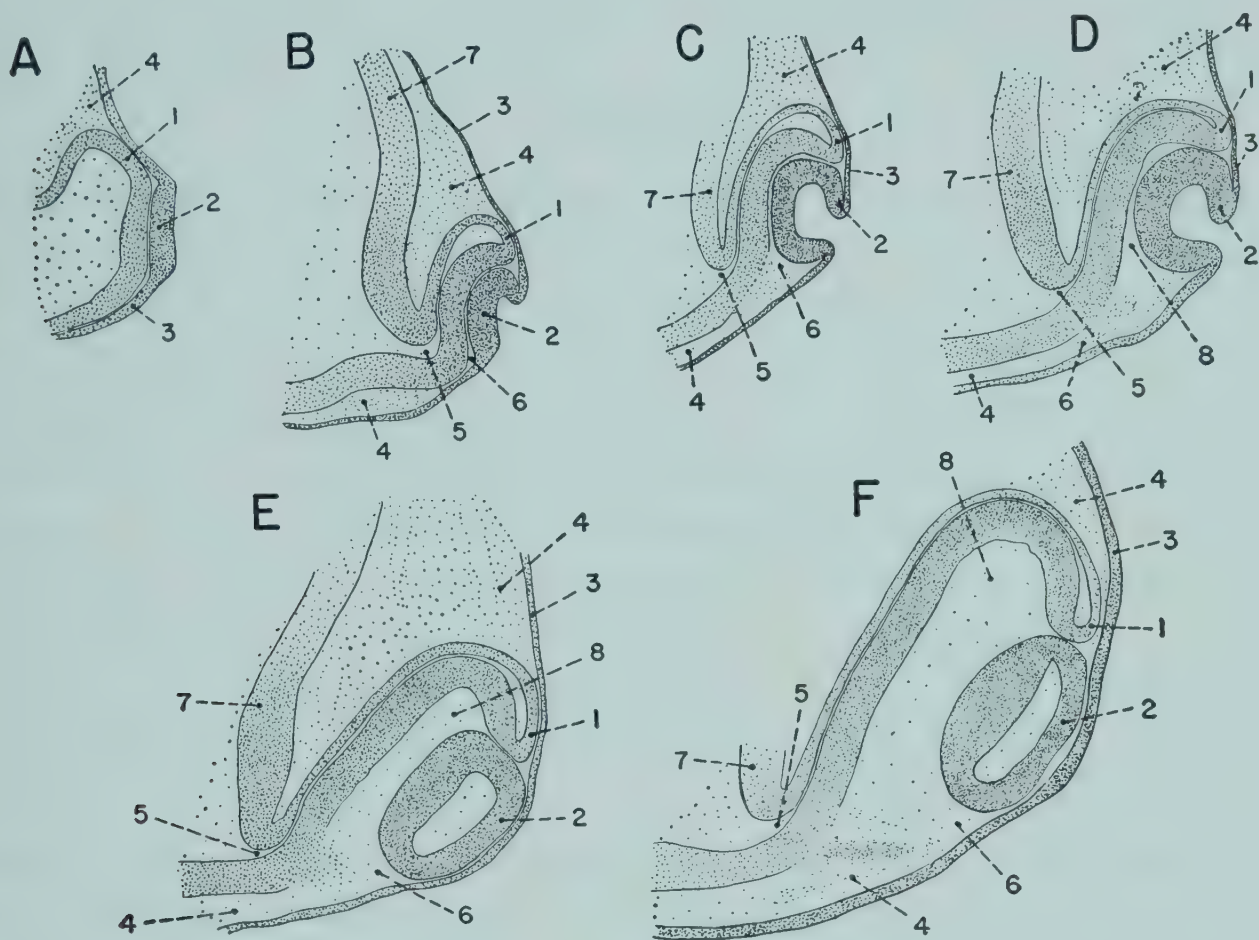
From its history during embryogeny, it is clear that the sensory portion of the eye is actually a part of the brain. The bilateral optic evaginations are apparent near the cranial end of the neural tube before the primary vesicles of the brain have differentiated (see Chapter 4). As the optic enlargements grow out, their medial portions are constructed to form the hollow optic stalks, by which they communicate with the brain, and their distal portions become the bulbous optic vesicles, also hollow. The outer, or lateral, wall of each optic vesicle then invaginates and obliterates the cavity of the vesicle. There is thus formed the double walled optic cup (or secondary optic vesicle), which is the primordial retina. The inner layer of the optic cup (originally the lateral wall of the optic vesicle) very soon becomes thicker than the outer layer. The latter is destined to become the pigmented layer of the retina, iris, and ciliary body.

The lens is derived from surface ectoderm, with which the optic vesicle comes in contact as it grows out. At the point of contact, the cells of the superficial layer become first elongated, then invaginated, and finally constricted off as a small hollow vesicle. The invagination of the lens vesicle coincides with the invagination of the optic cup. Several successive stages in the early development of the chick embryo's eye are shown in Fig. 148.

The inward growth of the lens ectoderm is at first directed somewhat dorsally (Lieberkühn, 1872; Kessler, 1877). Either for this reason, or



because the cells in the dorsal portion of the optic cup multiply the most rapidly, the primordial eyeball has an asymmetrical shape. It projects farther over the dorsal than the ventral surface of the lens vesicle (cf. Fig. 148) and is not, in fact, a cup at all during its early period. It is open laterally and ventrally where its double edges fail to come into apposition with each other, and this gap extends inward to the outer end of the optic stalk. In its lateral aspect, the optic cup appears to curve



**Fig. 148.** Successive stages in the early development of the eye in the avian embryo, seen in vertical section passing through the region of the chorioid fissure. (Redrawn with modifications after Kessler, 1877.)

A, at the 20-somite stage in the chick (*Gallus gallus*) embryo; B, 23-somite stage in the duck (*Anas platyrhynchos*) embryo; C, 27-somite stage in the chick embryo; D, the same, 30-somite stage; E, the same, 32-somite stage; F, the same, 39-somite stage. All  $\times 75$ .

1, optic vesicle or optic cup; 2, lens placode or lens vesicle; 3, surface ectoderm; 4, mesenchyme; 5, optic stalk; 6, region of chorioid fissure; 7, wall of forebrain; 8, vitreous chamber.

around the lens vesicle in the shape of a horseshoe. The part of the opening which is directly over the lens never closes and becomes the pupil. The rest of the opening, known as the chorioid fissure, gradually narrows as the optic cup grows and closes first in the portion intermediate between the lens and the optic stalk. The development of the pecten is intimately related to the closure of the chorioid fissure.

Except for the conjunctival epithelium (external to the cornea), which develops from the surface ectoderm over the lens, the remaining structures



of the eye—the iris, the ciliary body, the chorioid coat, the cornea, and the sclera—are of mesodermal origin. The sources of the vitreous humor and the material filling the aqueous chamber are still disputed.

Experiments have shown that the eye of the 36- to 72-hour chick embryo possesses the capacity for self-differentiation in high degree. When removed and grafted on the chorioallantoic membrane (*Hoadley, 1924*) or grown *in vitro* (*Strangeways and Fell, 1926*), the eye continues its histological differentiation, and the structural development of the retina and the lens proceeds in a manner closely approaching normal.

### The Lens

It is fairly well established that the lens is induced by the optic vesicle. Furthermore, induction of the lens is dependent upon contact between the optic vesicle and the surface ectoderm, since the lens fails to develop (in the chick embryo) if this condition is not met (*Danchakoff, 1926; Alexander, 1937; McKeehan, 1951*). The necessity for contact is explained if, as has been suggested, lens induction is initiated by a stereochemical reaction between molecules at the contiguous surfaces of the inducing and reacting tissues. The action of certain molecules at the surface of the optic vesicle possibly produces reorientation and fixation of complementary molecules at the basal surface of the ectodermal cells, which consequently undergo cytological changes and assume the typical aspect of lens placode cells (*McKeehan, 1951*).

In the early chick embryo, a fairly extensive area of surface ectoderm possesses the capacity to produce lens tissue. Optic vesicles placed in contact with ectoderm have induced lenses in various abnormal locations (*Danchakoff, 1926; Waddington and Cohen, 1936; Alexander, 1937; McKeehan, 1951*), even in extraembryonic regions (*Waddington and Cohen, 1936*). Alexander (1937) observed that lens-forming competence is exhibited at the 4-somite stage by ectoderm at eye, head-neck, and trunk levels but is no longer present in trunk ectoderm by the 8-somite stage. Deth (1940), however, found that this competence is retained by the ectoderm of the back until the 11-somite stage and is not lost by the head ectoderm and the presumptive corneal epithelium until the fourth and fifth incubation days, respectively. The capacity of the optic vesicle to induce a lens in the overlying ectoderm from 12-somite chick embryos cultured for 2 to 3 days was not impaired in experiments conducted by Langman (1956) but a direct contact between the optic vesicle and the overlying ectoderm was necessary. In experiments where contact was broken, there was merely a thickening of the presumptive lens ectoderm to several cell layers.

Retinal tissue can induce lens cells, even in a strange ectoderm, from the pre-somite to the 31-somite stage, at least (*Alexander, 1937*). Both



the sensory and the pigmented layers possess lens-inducing capacity (*Alexander, 1937; Amprino, 1951*).

There are a number of accounts in the literature of lens regeneration from the margins of the optic cup, although some of these reports are difficult to evaluate. Barfurth and Dragendorff (1902) destroyed the lens anlage and the edge of the optic cup of the 2.5- to 3-day chick embryo and found that both parts regenerated, the lens apparently from retinal tissue. Alexander (1937), who obtained similar results in transplants of retina, observed that lenses were produced only in the presence of epidermis and that they were often of dual origin, epidermal and retinal. On the other hand, Dorris (1938c) noted that lentoid bodies formed in cultures of isolated retinal tissue from 24- to 54-hour chick embryos. Deth (1940) stated that the margins of the chick embryo's optic cup can regenerate a lens up to the fourth incubation day and that the pigmented and sensory layers also have this ability, but only until the third and second days, respectively. It appears, too, that tissue from the margin of the optic cup can recover its early lens-forming ability after attaining an advanced stage of development. The epithelium of the 12-day chick embryo's iris, after dedifferentiating in culture, may transform itself into a lens, complete with capsule, when transplanted into a lensless eye (*Törö, 1931*). It is significant that corneal tissue does not do so. Lens determination probably begins during the 36- to 38-hour stage of cellular reorientation and extends beyond the 56-hour stage (*McKeehan, 1954*).

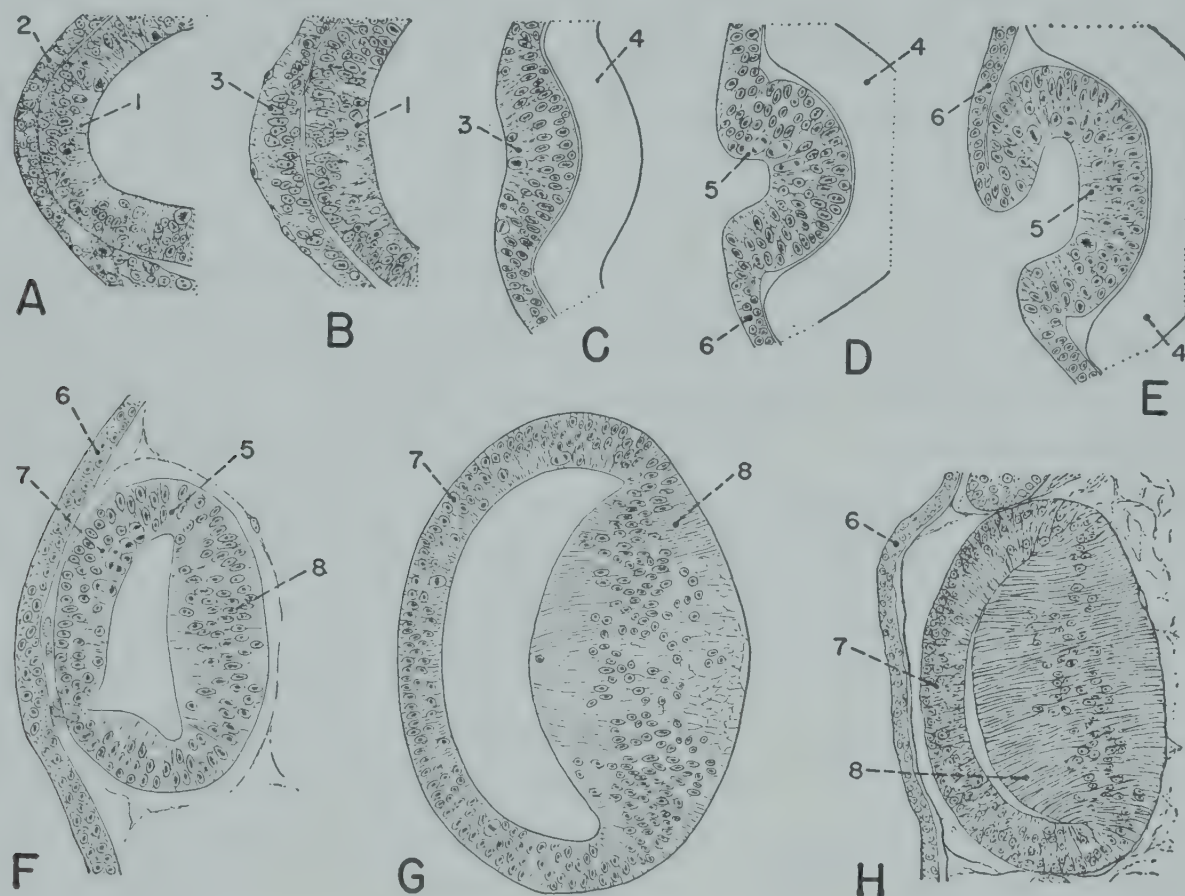
Precipitin tests have demonstrated the presence of adult lens protein in the lens vesicle of the 60-hour chick embryo, before the lens has separated from the ectoderm (*Cate, 1949; Cate and Doorenmaalen, 1950*). Therefore, it has been suggested that the chemical differentiation of the lens precedes its morphological differentiation and perhaps bears some causal relationship to the latter (*Cate and Doorenmaalen, 1950*).

The ectodermal thickening (the lens placode) which normally indicates the beginning of lens formation occurs directly over the area of contact with the optic vesicle. In actuality, it represents a condensation and contraction of the surface ectoderm and is produced by an increase in cell density (rather than in cell number), elongation of both cytoplasm and nucleus of individual cells, stratification of nuclei, and orientation of nuclei perpendicular to the surface. These changes occur between the 10- and 20-somite stages, when the lens ectoderm not only touches but adheres very firmly to the underlying optic vesicle (*McKeehan, 1951*).

The lens placode becomes visible at some time toward the end of the chick's second day of incubation (*Duval, 1889; Froriep, 1906; Slonaker, 1921*), at approximately the 20-somite stage (*Rabl, 1889b*); in the duck (*Anas platyrhynchos*), it has been seen at the 18-somite stage (*Rabl, 1889b*), and in the zebra parakeet (*Melopsittacus undulatus*) at the 23-



somite stage (Abraham, 1901). In the 21- to 25-somite chick and duck (*Anas platyrhynchos*) embryos (Duval, 1889; Rabl, 1889b; McKeehan, 1951), or the 25- to 26-somite zebra parakeet (*Melopsittacus undulatus*) embryo (Abraham, 1901), the lens ectoderm begins to invaginate to form the lens vesicle. Invagination appears to be due to an increase in tangential contraction at the outer ends of the lens cells (McKeehan, 1951).



**Fig. 149.** Successive stages in the development of the lens in the eye of the avian embryo, from a time prior to the formation of the lens placode to the period when the cavity of the lens vesicle is almost obliterated. (Redrawn with modifications A and B, after McKeehan, 1951; C, D, E, F, and G, after Rabl, 1889b; H, after Nordmann, 1938.)

A, 12-somite stage in the chick (*Gallus gallus*) embryo; B, the same, 19-somite stage; C, 20- to 23-somite stage in the duck (*Anas platyrhynchos*) embryo; D, the same, 23- to 25-somite stage; E, the same, 29-somite stage; F, the same, 35- to 36-somite stage; G, the same, 43-somite stage; H, from the chick embryo at 4 days' incubation. All  $\times 125$ .

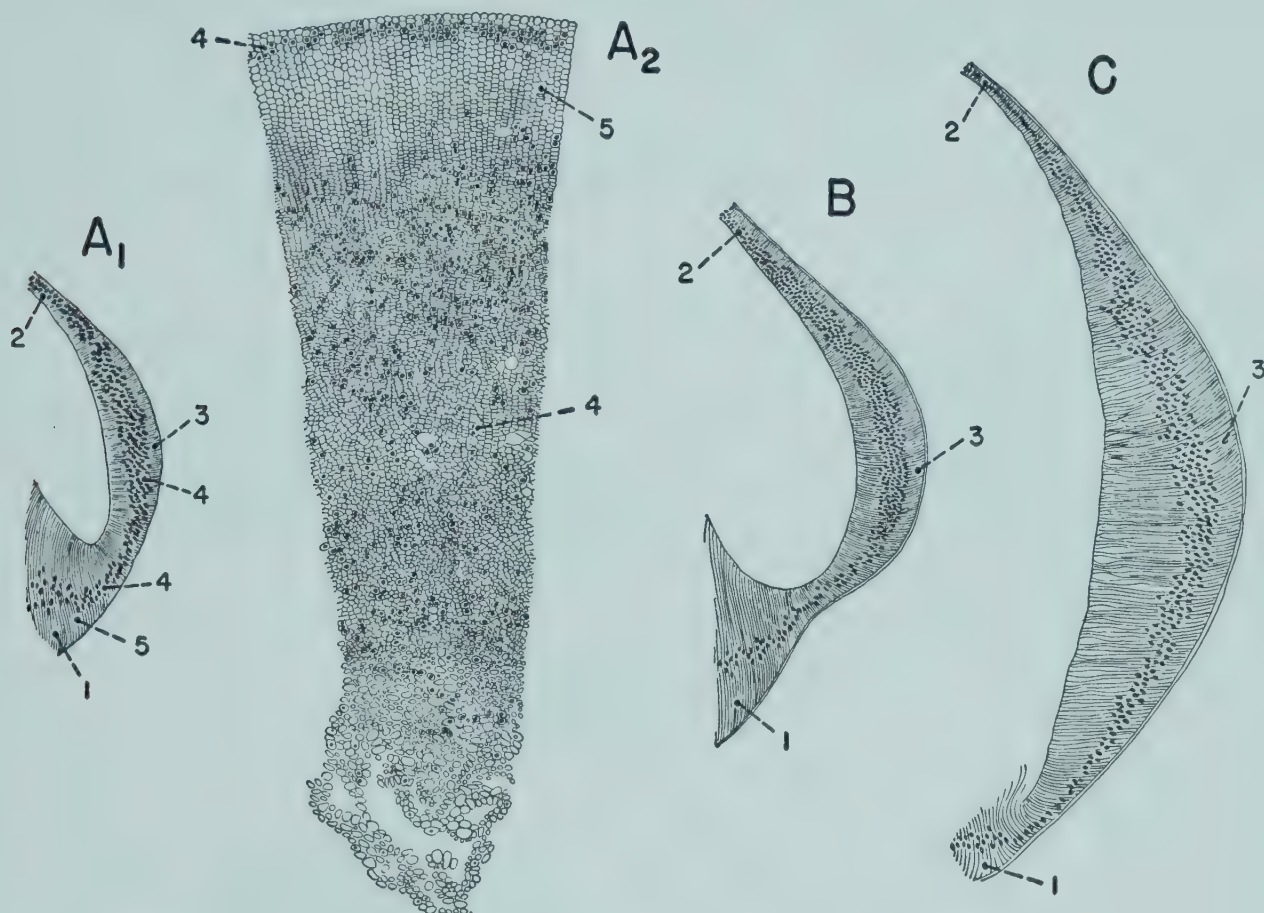
1, primary optic vesicle; 2, lens ectoderm; 3, lens placode; 4, invaginating optic vesicle; 5, invaginating or completed lens vesicle; 6, surface ectoderm; 7, future lens epithelium; 8, body of lens with differentiating fibers.

The pit formed by invagination closes and separates from the superficial ectoderm by the 35- to 36-somite stage (Rabl, 1889b), or at some time between the sixty-second and seventy-fourth hour of the chick's incubation period (Froriep, 1906; Slonaker, 1921). Figures 148 and 149 show the stages leading to the formation of the lens vesicle.

At the time of closure of the lens vesicle, or even earlier, the medial wall has already become slightly thicker than the lateral or distal wall (Kessler, 1877; Rabl, 1889b; Froriep, 1906; Slonaker, 1921). This difference is apparent in Fig. 149-F, and is due not only to increase in the total number



of cells in the medial wall but also to elongation of the individual elements, especially those located axially. The distal surface of the medial wall consequently starts to project into the lumen of the lens vesicle, and, as seen in the 43-somite duck (*Anas platyrhynchos*) embryo (Fig. 149-G), the pronounced bulging of the medial wall begins to obliterate the cavity (Rabl, 1889b). At this stage, also, the cell nuclei have become rearranged



**Fig. 150.** Details of histological development of the lens of the chick embryo's eye. (Redrawn with modifications after Rabl, 1889b.)

A<sub>1</sub>, B, and C, the annular pad after 8, 14, and 21 days' incubation, respectively, as it appears in meridional sections of the lens ( $\times 100$ ); A<sub>2</sub>, part of an equatorial section of the lens from an 8-day embryo ( $\times 275$ ).

1, body or lenticular portion of lens; 2, lens epithelium; 3, annular pad; 4, nuclei; 5, lens fibers.

so as to occupy a zone midway between the two surfaces of the medial wall, which may now be termed the lenticular portion of the lens. The medial surface of the lens body is flattened.

The cells of the lenticular portion continue to elongate and to become more fibrous (Fig. 149-H; Fig. 151). In the chick, they completely fill the lens cavity during the fourth day (Kessler, 1877, p. 9; Nordmann, 1938) or the fifth day (Froriep, 1906) of incubation. By this time, the chick's lens is no longer spherical; according to Froriep (1906), its equatorial diameter is now twice as long as its axial diameter (0.57 and 0.29 mm., respectively). The lens of the sparrow (*Passer domesticus*) embryo reaches this stage of development at about 2.5 days, although the sparrow's lens is more spherical than the chicken's throughout both embryonic and adult life (Slonaker, 1921).



Equatorial sections through the lens reveal that the fibers are at first hexagonal in cross section. As early as the sixth day of the duck's (*Anas platyrhynchos*) incubation period, and at least by the seventh day of the chick's, the fibers begin to rearrange themselves in radial rows and to become four-sided (Rabl, 1889b). This process begins peripherally and progresses toward the center of the lens (Fig. 150-A<sub>2</sub>).

The anterior (distal) wall of the lens (the lens epithelium) remains thin, but, as development progresses, it gradually increases in thickness in the equatorial region, where it joins the lenticular portion. This thicker equatorial part of the lens epithelium is the "annular pad" (Slonaker,

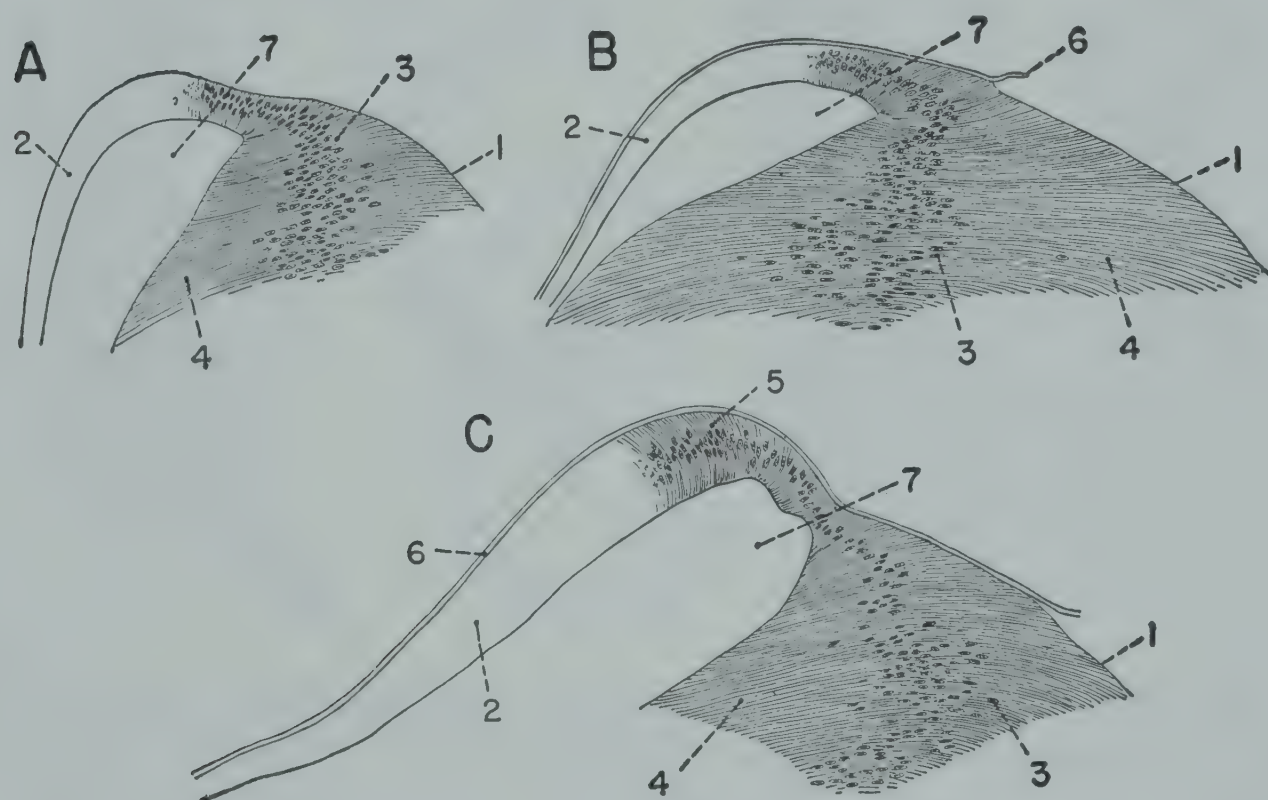


Fig. 151. Three stages in the differentiation of the lens in the eye of the duck (*Anas platyrhynchos*) embryo, as shown by meridional sections. (Redrawn after Rabl, 1889b.)

A, 7 days; B, 10 days; C, 18 days of incubation. All  $\times 150$ .

1, body of lens; 2, lens epithelium; 3, nuclei; 4, fibers; 5, annular pad; 6, lens capsule; 7, lenticular chamber.

1921) or "Ringwulst" of the German authors. It develops as the result of the lengthening of the cells (Slonaker, 1921). In the 8-day chick embryo (Fig. 150-A<sub>1</sub>), the 13- to 14-day duck (*Anas platyrhynchos*) embryo (Rabl, 1889b), and the 2-day-old sparrow (*Passer domesticus*) nestling (Slonaker, 1921), the lens epithelium again thins out in a narrow zone between the annular pad and the equator of the lenticular portion (Fig. 151). Beneath this region of thinner epithelium there is a small space, the lenticular chamber, which encircles the lenticular portion of the lens (cf. Fig. 151). The lenticular chamber is possibly formed as the result of pressure exerted by the accumulation of secretions from the cells of the annular pad (Slonaker, 1921). The fibrous cells of the annular pad, originally straight



and converging toward the center of the lens, now begin to curve (Fig. 150-B; Fig. 151-C), with their concave surfaces directed anteriorly (Rabl, 1889b). Their nuclei remain at their peripheral ends. A double row of nuclei is present in the thickest portion of the annular pad, a single row elsewhere in the lens epithelium (Fig. 150-C; Fig. 151-C). The lenticular chamber becomes continually more medial (or posterior) in position (Fig. 151-C) as the lenticular portion of the lens grows out distally (or anteriorly).

The most centrally located nuclei in the lenticular portion of the lens start to degenerate in the 8-day chick embryo (cf. Fig. 150-A<sub>2</sub>) and the 10-day duck (*Anas platyrhynchos*) embryo (Rabl, 1889b). Not all the nuclei have disappeared from the chick's lens by the hatching date, either from the lenticular portion or from the lens epithelium. In the sparrow (*Passer domesticus*), the nuclei of the lenticular portion disappear between the fourth and the eighth day after hatching (Slonaker, 1921).

### *The Lens Capsule*

The capsule surrounding the lens is generally believed to be the product of the lenticular cells (Kessler, 1877, p. 52; Rabl, 1889b; Nordmann, 1938). Kessler (1877, p. 52) stated that the outline of the lens placode is initially much sharper on the inner surface than on the outer, and Rabl (1889b) noted the presence of a capsule on the medial surface of the lens in the 43-somite duck (*Anas platyrhynchos*) embryo. According to Nordmann (1938), the lens capsule can be first seen as a darkly staining line bordering the medial wall of the lens vesicle shortly before separation of the latter from the ectoderm. Immediately underlying the early capsule there are clear spaces in the lens cells, producing a halo-like effect. The clear halo and the capsule extend around the entire lens before the lens cavity is completely obliterated.

It has been proposed that the distal surface of the lens is covered also by a membrane formed by the condensation of fibers lying in the anterior chamber of the eye. This membrane is said to appear in the chick embryo after 6 days of incubation. At first, it lies not only over the lens but also over a fibrous network which is connected with the mesenchyme of the future iris (Fig. 163-C and D). In the 15-day embryo, the portion of the membrane extending from the pupillary border to the lens has disappeared and the portion over the lens has fused with the underlying capsule of lenticular origin (Watzka, 1935).

### **The Retina**

The retina consists of two layers, the outer pigmented layer and the inner sensory layer. The latter contains several strata of cells of the same type as those found in other portions of the brain, of which the retina is really an integral part. Directly beneath the pigmented layer are the rods and cones,



which are specialized neurones and the sensory receptors of the eye. Portions of these cells project beyond the external limiting membrane of the sensory retina and extend into the pigmented layer. Proceeding toward the vitreous humor, one finds the following strata: the external nuclear layer, the external reticular layer, the internal nuclear layer, the internal reticular layer, the "ganglionic cell" layer, and the layer of optic nerve fibers. The nuclear layers are composed of the cell bodies of neurones and the reticular layers consist of fibers arising from them. The only fibers which leave the retina are those of the optic nerve, given off by the ganglionic cells. The nervous elements of the retina lie among supporting cells, the fibers of Müller, and neuroglia elements.

In birds, the retina is considerably thicker than in many animals, the retinal elements are fine and closely arranged, and the various strata of the sensory layer are more sharply defined than in any other vertebrate (Wood, 1917, *p.* 16). Among various avian species, there are differences in the structure of the retina, chiefly with respect to the numerical ratio between rods and cones, and the depth and position of the fovea.

The location and destiny of the retinal material is already determined in the chick embryo of 2 or 3 somites. If the early optic evaginations are removed, there is no regeneration of the eye from the prosencephalic region (Amprino, 1951). The sensory and pigmented layers of the retina are also determined at an early stage, for, at the 6- to 8-somite stage, the presumptive material of one cannot be reconstituted at the expense of the presumptive material of the other (Amprino, 1951); however, each retains the capacity to regenerate its own tissue up to the 12- or 14-somite stage. The pigmented layer can differentiate when removed from its normal relationship with the sensory layer, but there is some evidence that differentiation of the sensory layer depends upon the presence of the pigmented layer.

The development of the retina may be said to begin with the invagination of the optic vesicle to form the optic cup. A slight depression is detectable in the lateral wall of the optic vesicle almost as soon as the lens placode forms (cf. Fig. 148-A; Fig. 149-B); that is, at about the 20-somite stage, in the chick (Rabl, 1889b). Invagination of the optic cup and obliteration of the cavity of the optic vesicle is complete by the 30-somite stage, or shortly before the lens vesicle closes (cf. Fig. 148-D).

Almost as soon as the optic cup starts to invaginate (cf. Fig. 148-B), the future sensory layer begins to increase in thickness (Koganei, 1884), especially in the central or axial region (Slonaker, 1921), and to extend its surface area (Cameron, 1902; Weyssse and Burgess, 1906). The early growth of the inner layer of the retina is due to the mitotic division of its outermost nuclei (those closest to the pigmented layer), which constitute a germinal layer. Some cells migrate toward the vitreous humor to differentiate, and others remain on the surface to continue the process of pro-



liferation. Distention of the optic cup by the vitreous humor is a factor in the growth (see Appendix, Table V) of the eye (the volume of which increases about 500 times between the fourth and ninth days), for expansion of the retina permits a larger number of cells to remain in the proliferative zone than could do so in an unstretched retina. Experimental evidence of the influence of the vitreous humor is provided by the great reduction in the growth rate of the eye after drainage of its contents on the fourth day. In such a collapsed eye, the sensory layer of the retina becomes wrinkled and folded, and, although undersized, thus demonstrates that it possesses a greater capacity for growth than the pigmented layer, which remains smooth (*Weiss and Amprino, 1940*).

### *The Inner or Sensory Layer*

According to Weyssse and Burgess (1906), there are three periods in the histological development of the chick embryo's retina: (1) a period of cell multiplication, from the second to the eighth incubation day; (2) a period of cellular readjustment, from the eighth to the tenth day; and (3) a period of final differentiation from the tenth day to the end of incubation.

The development of the various strata begins at the axis of the retina and proceeds toward the margin. The first step is the differentiation of neuroblasts, from which are derived the cells of the ganglionic and inner and outer nuclear layers. Neuroblasts and nerve fibrils are already present at the end of the third day of incubation (see Chapter 4), and neurofibrils are found from the axis to the equator of the eye in the 5-day chick (*Tello, 1923*). The earliest neuroblasts to form are for the most part destined to contribute to the ganglionic cell layer, which, in the 4-day chick embryo, can be seen immediately external to the nerve fiber layer arising from it. Elongated supporting cells, which differentiate the fibers of Müller, are mingled with the ganglion cells. After 5 to 7 days of incubation, the fusion of the internal ends of the Müller's fibers produces the internal limiting membrane of the retina (*Koganeï, 1884*). The ganglion cell layer now consists of one or two rows of cells, and the remaining nuclei are eight to twelve rows deep (Fig. 152-A).

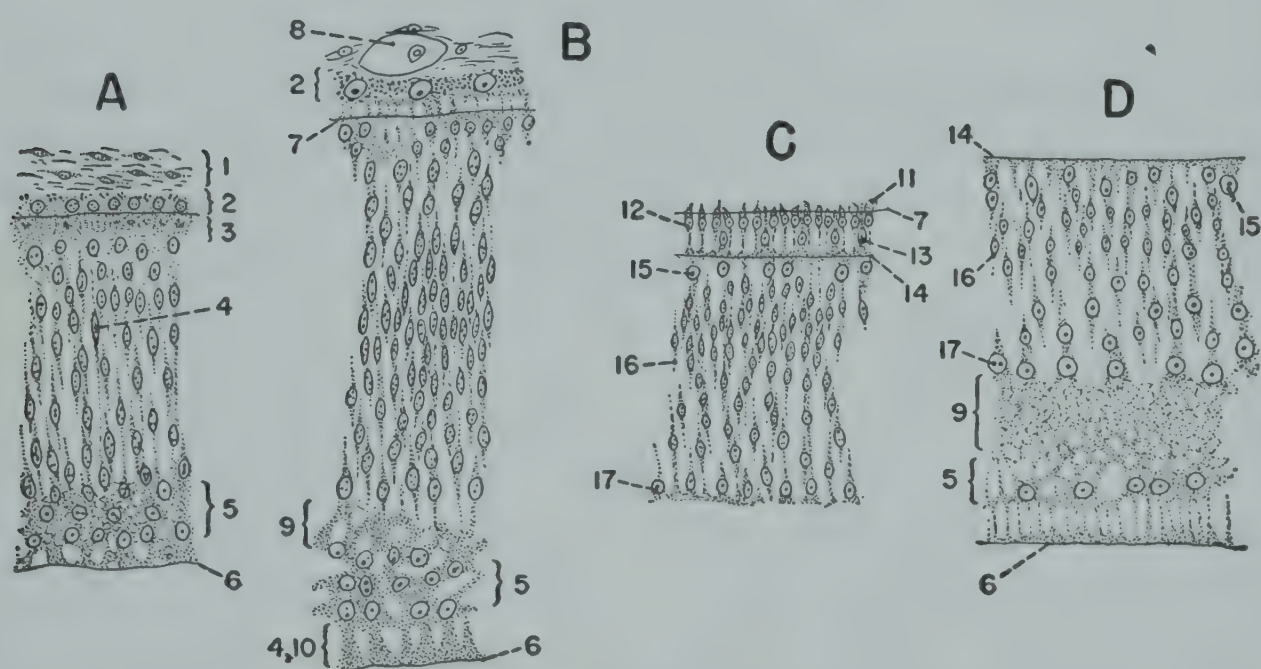
The first indication of the inner reticular layer appears in the 7- to 8-day embryo (Fig. 152-B) external to the ganglionic layer (*Koganeï, 1884*). The outer reticular layer differentiates a day or two later and is much narrower (*Cameron, 1902*). Simultaneously, the inner nuclear layer becomes apparent; it consists of the nuclei between the two reticular layers.

In the retina of the 8-day chick embryo, the ganglionic and inner reticular layers are present everywhere in the retina except in the marginal one fourth, that is, in the part closest to the lens (*Cameron, 1902*). The transition between the marginal portion and the much thicker fundal portion is quite sudden. The zone of transition is known as the ora serrata (cf. Fig.



161-A and B), or ora terminalis (*Lenhossék, 1911*), since it has no serrations, in birds.

After 8 or 9 days of incubation, the ganglionic cell layer is three nuclei deep, and the inner nuclear layer contains fourteen or more rows of nuclei (*Koganeï, 1884; Weyssse and Burgess, 1906*). The retina grows thicker until the ninth incubation day (*Koganeï, 1884*). After this time, its cells undergo a rearrangement which continues its expansion but causes a decrease in thickness. Between the ninth and the tenth or twelfth day of incubation, the cells of the ganglionic cell layer become aligned in one row instead of three. The inner nuclear layer becomes narrower as its cells close up their ranks; the inner reticular layer, however, is widened somewhat.



**Fig. 152.** Histological development of the retina in the eye of the chick embryo, shown in semidiagrammatic representations. (Redrawn with modifications after *Weyssse and Burgess, 1906*.)

A, at 7 days; B, at 8 days; C, at 10 days; D, at 16 days of incubation. All  $\times 230$ .

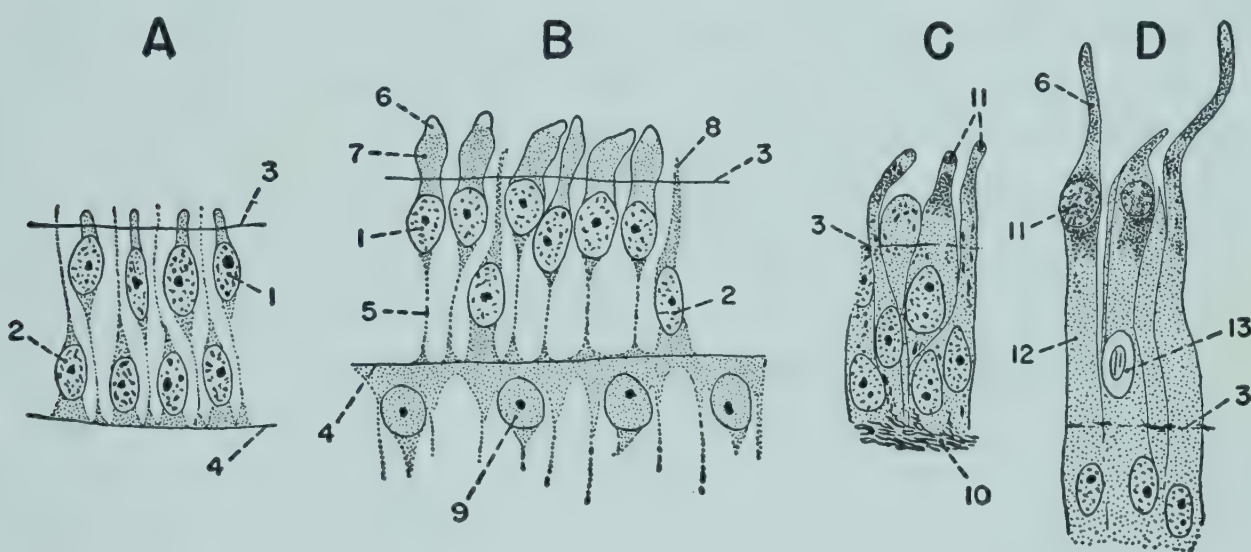
1, chorioid coat; 2, pigment layer; 3, germinal layer; 4, radial fiber of Müller; 5, ganglion cell layer; 6, internal limiting membrane; 7, external limiting membrane; 8, blood vessel; 9, inner reticular layer; 10, nerve fiber layer; 11, cone; 12, cone nucleus; 13, rod nucleus; 14, Henle's membrane; 15, outer horizontal cell; 16, bipolar cell; 17, inner horizontal cell.

The three innermost rows of cells in the inner nuclear layer then differentiate as the inner horizontal or amacrine cells (correlation neurones), and the outermost cells of the inner nuclear layer become the basal or outer horizontal cells (*Fig. 152-C*). Some of the intermediate cells become supporting cells giving rise to the fibers of Müller. External to the inner nuclear layer, the membrane of Henle is formed (*cf. Fig. 152-C*) as the cytoplasm of the outer horizontal cells (together with the ends of the Müller's fibers and of the remaining cell processes) becomes pressed against the cytoplasm of the rod and cone cells (*Weyssse and Burgess, 1906*).

The rods and cones have also started to differentiate during the period



from the tenth to the twelfth day of incubation. Their nuclei constitute the outer nuclear layer. In the 9.5- to 10-day chick embryo, this layer consists of two rows of nuclei, of which the outer are the cone nuclei (Koganeï, 1884). The cytoplasm of both cell types is elongated, extending from the membrane of Henle on one side out through the external limiting membrane on the other side (Fig. 153-A). The cytoplasm of each rod cell tapers from a broad base at the membrane of Henle to a fine point, capped by a globular swelling at its external end (Koganeï, 1884; Cameron, 1902). In the cone cells, on the other hand, the internal portion of the cytoplasm is drawn out into a finely attenuated stalk flattened at its base against the membrane of Henle, and the portion outside of the external limiting mem-



**Fig. 153.** Successive stages in the development of the rods and cones of the chick embryo's retina. (Redrawn with modifications A and B, after Weyssse and Burgess, 1906; C and D, after Leplat, 1914.)

A, at 10 days of incubation; B, at 11 days of incubation; C, at 16 days of incubation; D, 1 day after hatching. All  $\times 900$ .

1, cone nucleus; 2, rod nucleus; 3, external limiting membrane; 4, Henle's membrane; 5, cone fiber; 6, outer segment; 7, granular zone of cone; 8, primordium of rod; 9, outer horizontal cell; 10, outer reticular layer; 11, oil droplet; 12, internal segment; 13, ellipsoid body.

brane is at first blunt and then becomes conical (Weyssse and Burgess, 1906). Granules regarded by Leplat (1912, 1914) as derived from mitochondria are present in the protruded portion of both rod and cone cells (Fig. 153-B), and on approximately the twelfth day an oil droplet is formed in close proximity to the granules. The oil globule attains the outer extremity of the rod or cone (Fig. 153-C) by the sixteenth day (Leplat, 1914) and becomes red or yellow by the eighteenth day (Cameron, 1902). On the fourteenth day (Cameron, 1902)—or much later, toward the end of incubation (Leplat, 1914)—spherical structure (the ellipsoid body) appears between the oil droplet and the external limiting membrane, in the portion of the protruding cytoplasm known as the internal segment (Fig. 153-D). Cameron (1902) has stated that the ellipsoid body originates internal to



the membrane and passes through it to attain its final position. According to Leplat (1914), the external segment of cytoplasm, peripheral to the oil globule, is represented on the sixteenth day by a single filament extending beyond the oil droplet, but after the eighteenth day this filament becomes gradually surrounded by cytoplasm which contains granules of mitochondrial origin and which eventually takes on a transversely striated appearance. Cameron (1902), however, believed that the external segment is composed of the first cytoplasm to be protruded, and the internal segment of cytoplasm protruded later. In the sparrow (*Passer domesticus*), the rods and cones require several days after the hatching date in which to attain the stage of development found in the chick at the end of incubation (Slonaker, 1921).

**The Fovea.** The fovea (the small pit in the sensory layer of the retina where vision is most acute) appears late in development at the point where differentiation of the sensory layer began. The fovea occupies the center of the small, circular or oval area centralis, which is somewhat thicker than the rest of the retina. The area centralis starts to develop at approximately the time that the layers of the retina begin to differentiate. It is formed largely by a local increase in the thickness of the inner and outer nuclear layers (Chievitz, 1890). It is possible that this localized thickening is the result of the richer blood supply provided by an earlier and marked increase in thickness of the chorioid coat in the same region (Slonaker, 1921). The fovea is produced by a radial migration of the cells of the various retinal layers away from the center of the area centralis. In birds hatched with their eyes closed—such as the sparrow, *Passer domesticus* (Slonaker, 1921), the European rook, *Corvus frugilegus*, the tern, *Sterna* sp., and the pigeon, *Columba livia* (Chievitz, 1890)—the area centralis and the fovea do not start to develop until the hatching date or later, and the most rapid differentiation of the fovea occurs after the eyes have opened. In the tern, there are two foveae, which develop simultaneously (Chievitz, 1890).

### *The Outer or Pigmented Layer*

The outer layer of the optic cup develops into the pigmented retinal epithelium, which extends to the pupillary margin and forms part of the ciliary body and the iris. This layer is much thinner than the sensory layer, even in the early stages of development. It is thinnest in the axial region (Slonaker, 1921), where, by the end of the chick's third incubation day, it consists of only one layer of cells. At the margin of the optic cup it is composed of two layers of actively dividing cells until the eye ceases to increase in size (Weysse and Burgess, 1906).

Before the invagination of the optic cup, the cells of the future pigmented layer appear distinct from those which are to become visual elements, since they possess larger nuclei and less cytoplasm than the latter



(Smith, 1920). Also, a few small, gray pigment granules have been distinguished in their cytoplasm at this time, in specially prepared specimens (Smith, 1929). The size, number, and color intensity of the granules increases rapidly until, at 66 hours, a few black granules of maximum size are present. The period from 64 to 72 hours of incubation has usually been mentioned as that in which the pigmentation of the retina begins in the chick (Weyssse and Burgess, 1906; Tello, 1923). Retinal pigment has been observed to appear initially on the fourth incubation day in the sparrow (*Passer domesticus*) embryo (Slonaker, 1921) and on the fifth day in the embryo of the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901). According to Coulombre (1955) there is a marked decrease in mitotic activity and the appearance of melanin granules in the pigmented epithelium of the chick retina during the fourth and fifth incubation days. At this time the neural retina is characterized by active cellular proliferation and increase in retinal area and thickness.

There are conflicting accounts regarding the direction in which pigmentation proceeds in the retina. Leplat (1914) observed that the development of pigment progresses from the fundus to the pupillary border of the retina in the chick embryo, whereas Slonaker (1921) stated that it takes place in the opposite direction (from the retinal margin to the optical axis) in the sparrow (*Passer domesticus*). Both authors, however, agreed that the first pigment granules to appear vary in their location within the cell in different parts of the retina. They are found at the inner end of each cell in the marginal portion and at the outer end in the fundal portion. Harrison (1951), investigating pigment formation of embryonic chick retina *in vitro*, concluded there is an inherent tendency for right eyes to pigment first.

The origin of the retinal pigment is disputed. According to Szily (1911), colorless granules are extruded from the nucleus and become pigmented later through the action of enzymes; Leplat (1912) and Luna (1913), however, suggested that the chick's retinal pigment is derived from mitochondria. There is little evidence in support of either theory. Smith (1920) has concluded that the pigment in the retina of the chick arises in the cytoplasm of the cell by a process which takes place in two stages: (1) the formation of a colorless chromogen; (2) the production of color in the chromogen.

### *Physiology of the Retina*

It is of interest to mention that the acetylcholine content and the cholinesterase activity of the chick embryo's retina increase steadily from the eighth to the nineteenth day of incubation and then rise sharply (Lindeman, 1947). Alkaline phosphatase activity also increases abruptly between the sixteenth and nineteenth days, coincident with a slight eleva-



tion in acid phosphatase activity (*Lindeman, 1949*). These observations are consistent with the concept that acetylcholine and cholinesterase play a specific role in the functional activity of the nervous system, and that acid and alkaline phosphatase are concerned with the histochemistry of differentiation. It appears that the neural units of the retina have matured by the nineteenth day and are capable of conducting impulses, for the constrictor pupillary reflex can first be elicited at that time (*Lindeman, 1947*). *Tamiya (1927a)* observed a sudden increase in the metabolism (anaerobic glycolysis) of the retina between the eighteenth and the nineteenth day.

### The Pecten and the Closure of the Chorioid Fissure

The pecten varies greatly in size and shape in different species of birds. In general, however, it is a thin, darkly pigmented lamina, folded upon itself like a fan, which projects into the vitreous body from the ventral surface of the eye. The pecten may contain from five to thirty folds and may be of any length from very short to sufficiently long to reach the lens. It consists largely of a vascular network supported by pigmented connective tissue (glia cells) and is covered by a membrane that is continuous with the inner limiting membrane of the retina. The pecten is probably nutritive in function (*Husen, 1913*) and perhaps provides protection against strong light. It has been suggested, also, that it plays a part in accommodation (*Rabl, 1889b*) and that it is a sense organ (*Franz, 1910*).

The development of the pecten and the closure of the chorioid fissure, two simultaneous and closely connected processes, have not been described similarly by all authors. Some have stated that the fissure closes first at its distal end, and many have regarded the pecten as a structure of exclusively mesodermal origin (*Huschke, 1831; Remak, 1851; Lieberkühn, 1872; Mihalkovics, 1873; Kessler, 1877, p. 67; Froriep, 1906; Slonaker, 1921*). Others have reported that closure of the fissure starts at the proximal end (*Szily, 1922*) and between the optic stalk and the pupil (*Mann, 1921; Lindahl and Jokl, 1922*), and a number of investigators have declared that the pecten, or at least its supporting framework, is derived from ectoderm (*Nussbaum, 1901a; Parreidt, 1901; Bernd, 1905; Husen, 1913; Lindahl and Jokl, 1922; Szily, 1922*). The highly vascularized condition of the adult pecten indicates that mesodermal tissue participates in the formation of the organ and eventually predominates (*Mann, 1924; Pasquini, 1925*).

The open chorioid fissure extends from the optic stalk to the pupillary border, where its edges diverge somewhat. Figure 157-A shows this stage in the duck (*Anas platyrhynchos*) embryo of 102 hours' incubation. Approximately at the time the lens vesicle closes—or after about 58 hours' incubation, in the chick (*Husen, 1913*)—mesodermal elements penetrate through the fissure and project slightly into the vitreous chamber. In the



79-hour chick embryo, a blood vessel (the “arteria cupulae opticae”) originating in the vascular plexus ventral to the eye is found in the mesodermal tissue included between the lips of the fissure. The vessel enters near the proximal end of the fissure and leaves near the distal end (Kessler, 1877; Lindahl and Jokl, 1922; Szily, 1922; Mann, 1924).

According to the account given by Szily (1922), the closure of the chorioid fissure in the chick embryo begins at the proximal end after about 3.5 days' incubation. About 12 hours later, the fissure closes between the pupillary border and the point where the intraocular blood vessel leaves the eye (Fig. 154-A). Fusion of the edges of the fissure is followed, in this region, by complete separation of the two retinal layers. Closure of the intermediate portion of the fissure, which contains the mesodermal lamella between its lips, begins after about 5 days' incubation immediately proxi-

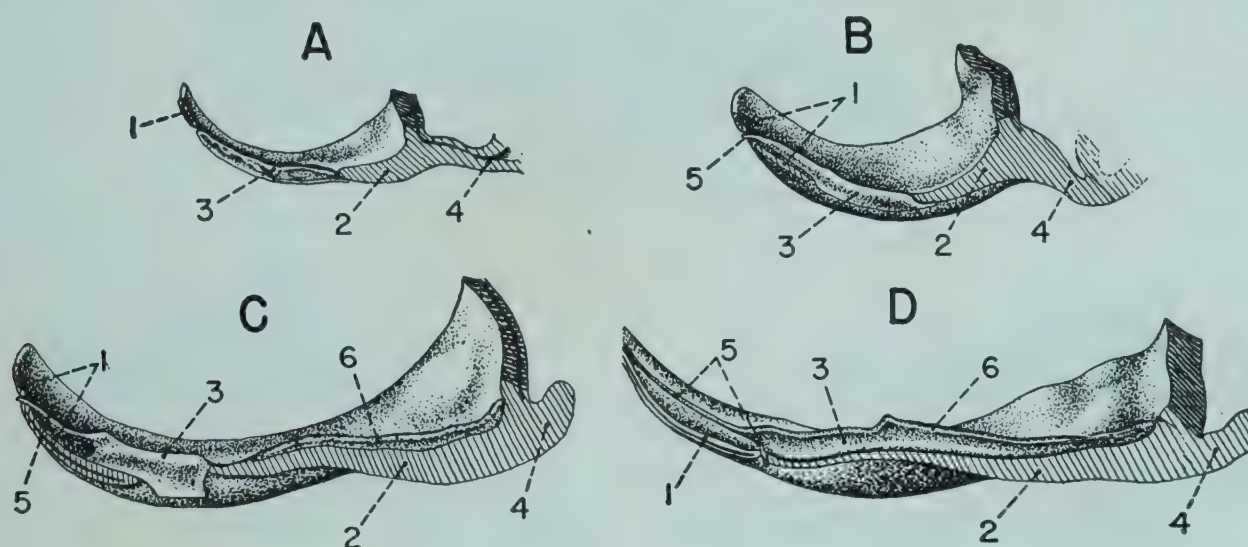


Fig. 154. Several stages in the closure of the chorioid fissure in the eye of the chick embryo (semidiagrammatically represented), in approximately sagittal sections of the ventral portion of the eye. (Redrawn after Szily, 1922.)

A, 4 days 18 hours; B, 5 days 6 hours; C, 6 days 18 hours; D, 7 days 15 hours.

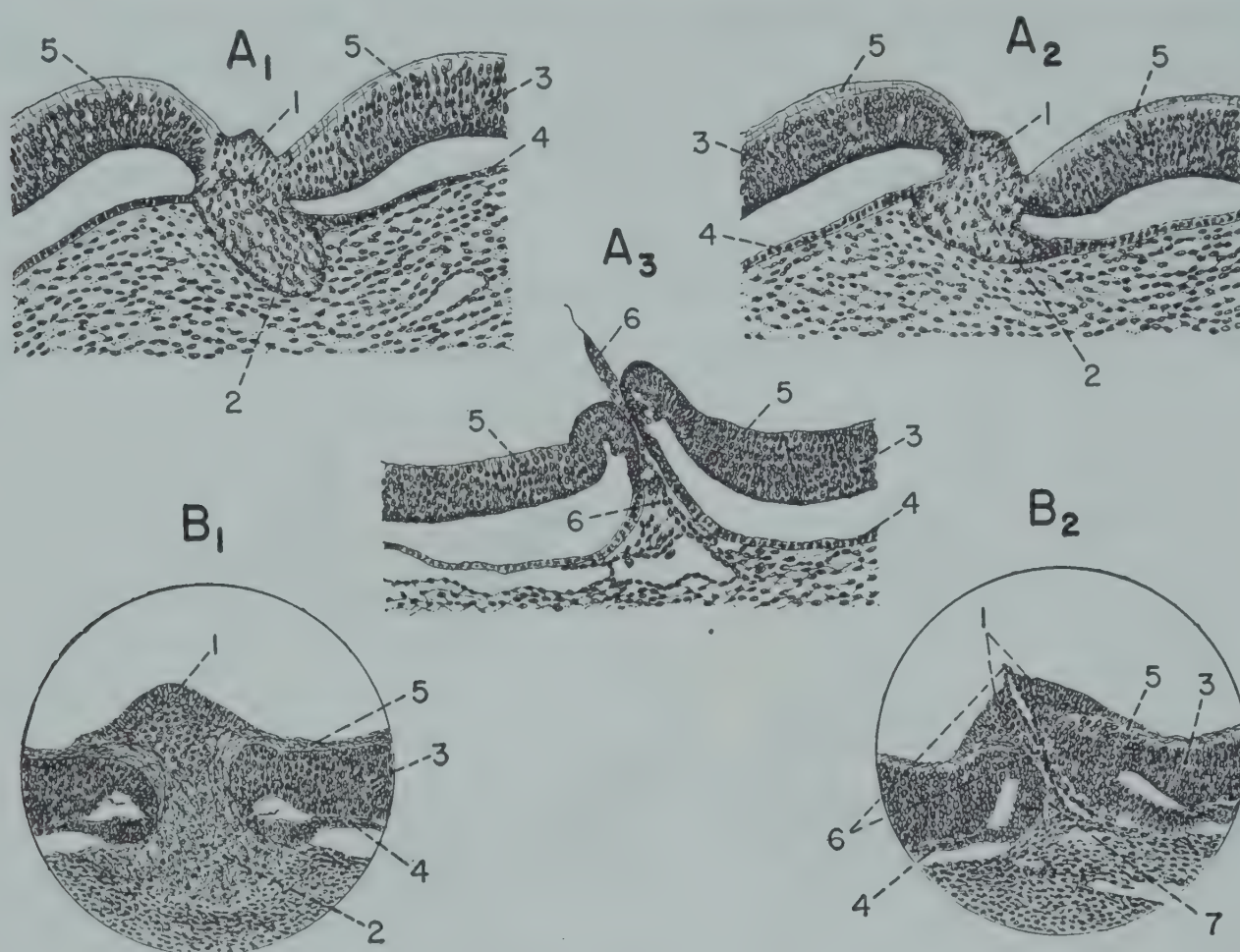
1, distal closed portion of chorioid fissure; 2, proximal closed portion of chorioid fissure (cauda of optic nerve); 3, mesodermal tissue; 4, optic stalk; 5, blood vessel; 6, pecten.

mal to the point of exit of the blood vessel and progresses toward the equator of the eye (Fig. 154-B). The edges of the fissure fuse below the mesodermal tissue, and, as in the proximity of the pupillary border, the retinal layers separate. The mesodermal tissue isolated inside the eye gradually becomes reduced until it consists merely of the intraocular blood vessel, which, in the 6.75-day embryo, is free within the vitreous chamber for a short distance before passing out through the wall of the eye (Fig. 154-C; cf. Fig. 160-A<sub>2</sub>).

The closure of the proximal section of the chorioid fissure occurs quite differently and is correlated with the formation of the elongated cauda of the optic nerve, or its “insertion” (which is actually its egress), and with the simultaneous formation of the pecten. The process of closure progresses lensward from the point where the optic stalk joins the eye. At this point



there is a fold of the inner retinal layer extending slightly into the lumen of the optic stalk. Fusion begins at the apex of the fold after about 3.5 days' incubation. Through active cellular proliferation, the fold then bridges the lumen of the optic stalk, thus providing a pathway for the exit of nerve fibers from the retina. A moderate number of fibers are seen leaving the eye by this route after 4.5 days' incubation. Less than a day



**Fig. 155.** Stages in the development of the pecten and the cauda of the optic nerve in the eye of the avian embryo. (Redrawn with modifications A, after Szily, 1922; B, after Lindahl and Jokl, 1922.)

A<sub>1</sub>, a section through the ventral wall of the 5-day chick (*Gallus gallus*) embryo's eye, not far from the optic stalk and transecting the region of the closed chorioid fissure; A<sub>2</sub>, a section taken somewhat more distally from the same eye; A<sub>3</sub>, a more distal section taken in the region of the still open chorioid fissure; B<sub>1</sub>, a section from the proximal part of the closed chorioid fissure of the 8.75-day duck (*Anas platyrhynchos*) embryo's eye; B<sub>2</sub>, a more distal section from the same eye, at the point close to the open portion of the chorioid fissure. All  $\times 65$ .

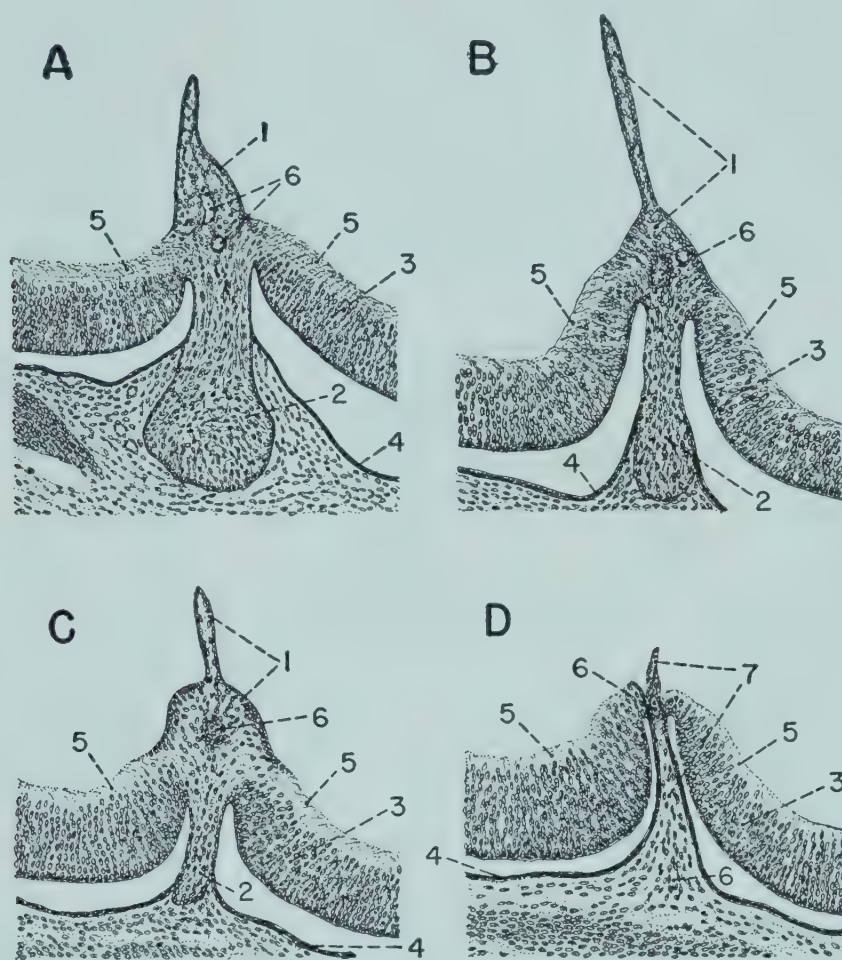
1, primordium of pecten; 2, cauda of the optic nerve; 3, inner or sensory layer of the retina; 4, outer or pigmented layer of the retina; 5, layer of nerve fibers in the retina; 6, mesodermal tissue; 7, nerve fibers leaving the retina.

later, the pecten projects into the vitreous humor as a low ridge along the line of fusion of the chorioid fissure (Fig. 155-A<sub>1</sub> and A<sub>2</sub>).

As the closure of the chorioid fissure progresses distalward from its proximal end, the cauda of the optic nerve, continuous with the optic stalk, forms an increasingly long ridge on the ventral surface of the eye (Fig. 154; Fig. 155; Fig. 156). The growing pecten produces a coextensive and continually more prominent ridge on the internal surface (Fig. 155;



Fig. 156). Grossly, the primordial pecten of the 6- to 7-day embryo seems to be continuous distally with the mesodermal lamella, which forms a similar ridge on the inner surface of the eye in the region where the fissure is still open (cf. Fig. 154-C and D). Szily (1922), however, found no cellular continuity between the two structures, the mesodermal tissue seemingly retreating before the advance of the forming pecten and cauda. The



*Fig. 156.* The structures found in the region of the chorioid fissure in the chick embryo's eye after 7 days 15 hours of incubation; as seen in sections. (Redrawn with modifications after Szily, 1922.)

A, a section taken across the closed portion of the chorioid fissure, not far from the optic stalk, and transecting the pecten and the cauda of the optic nerve; B, C, successively more distal sections parallel to A; D, a still more distal section, taken through the small, still open portion of the chorioid fissure which admits a sheet of mesodermal tissue into the vitreous chamber of the eye. All  $\times 60$ .

1, primordium of pecten; 2, cauda of the optic nerve; 3, inner or sensory layer of the retina; 4, outer or pigmented layer of the retina; 5, layer of nerve fibers in the retina; 6, blood vessel; 7, mesodermal tissue.

edges of the open portion of the fissure are inverted (Fig. 155-A<sub>3</sub>; Fig. 156-D).

After 7 days 15 hours of incubation (Fig. 154-D), the opening through which the intraocular blood vessel gains access to the eye has closed from either end to such an extent that it is barely larger than necessary to permit the entrance of the vessel. The mesodermal lamella has disappeared except for a small remnant within this opening. The cauda of the optic nerve has extended itself distally through two thirds of the ventral circumference



of the eye. Its more distal portion lies within a groove formed on the exterior of the eye by the inverted outer retinal layer (Fig. 156-B and C). The pecten, located above the optic nerve fibers coming from either side of the closed fissure, gains in height from its proximal end, where it forms a high triangle in cross section, to its distal end, where it is a thin plate rising from a broader base (Fig. 156-A, B, and C). It is composed of loose syncytial tissue, and a blood vessel is visible in its basal portion.

A few additional details, not altogether reconcilable with Szily's findings, were provided by Mann (1924). This observer stated that the inner retinal layer is both everted and inverted along the edges of the proximal portion of the fissure, so that a double ridge is produced on the external and the internal surface of the eye. In the middle portion of the fissure, there is

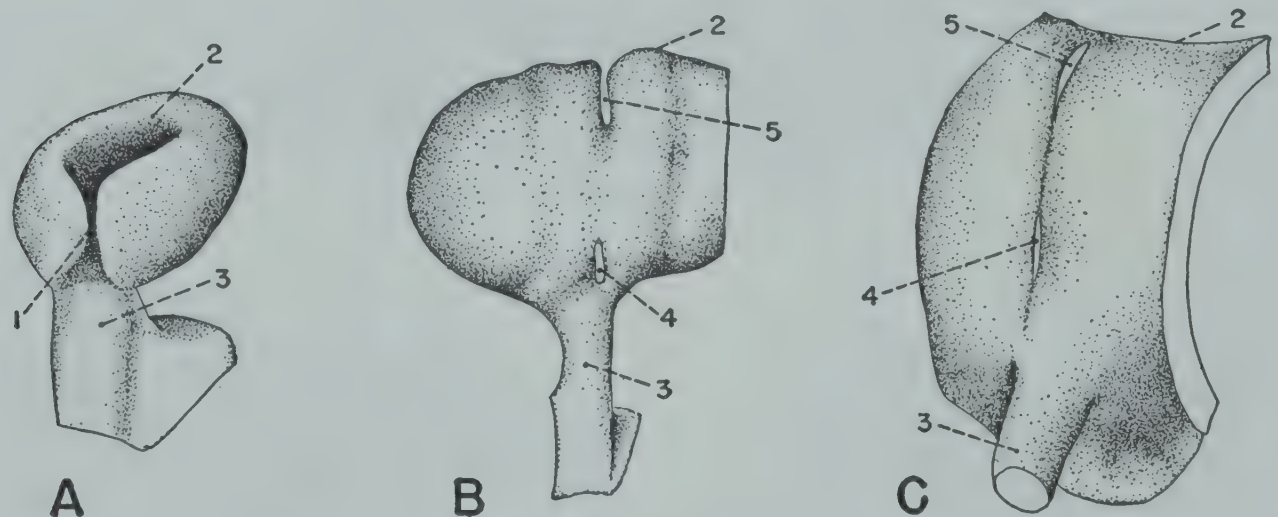


Fig. 157. Three stages in the closure of the chorioid fissure, shown in external views of the duck (*Anas platyrhynchos*) embryo's eye. (Redrawn after Lindahl and Jokl, 1922.)

A, at 4.25 days; B, at 5.5 days; C, at 6.25 days of incubation. All  $\times 40$ .

1, chorioid fissure; 2, margin of optic cup (pupillary margin); 3, optic stalk; 4, proximal unclosed portion of the chorioid fissure; 5, distal unclosed portion of chorioid fissure.

no eversion of the retina, and hence no external ridge; and in the distal portion the retina is neither inverted nor everted. The edges of the fissure merely come into apposition in the latter region, where this author indicated that closure begins. Proximally, fusion of the lips of the fissure starts internally and progresses toward the exterior of the eye, thus occurring first where the retina is inverted. The fused inverted lips of the fissure form the primitive pecten. The optic nerve fibers penetrate through the retina beneath the primitive pecten and make their exit to the optic stalk along the fused everted lips of the fissure. As the proximal end of the fissure closes in this manner, it elongates because of the relatively very rapid growth of the part of the eye in which it lies. The apparent distalward displacement of the mesodermal lamella, noted by Szily (1922), is due to the same cause.



A report essentially in agreement with that of Szily (1922) was made by Lindahl and Jokl (1922), who conducted an extensive investigation on embryos of the crested grebe (*Podiceps cristatus*), the duck (*Anas platyrhynchos*), the sparrow (*Passer domesticus*), and the chick. These authors stated, however, that the fissure closes first in its midportion and then at the pupillary border (Fig. 157-B and C), leaving open a fairly long proximal section and a small distal slit through which passes the arteria cupulae opticae (Fig. 158-A). As the proximal opening elongates during the chick's fifth, sixth, and seventh incubation days, the inversion of its edges becomes especially pronounced. The nasal edge turns in somewhat more markedly than the temporal edge, is larger, and slightly overlaps it (Fig. 158-B). The cells of both edges retain their primitive undifferentiated

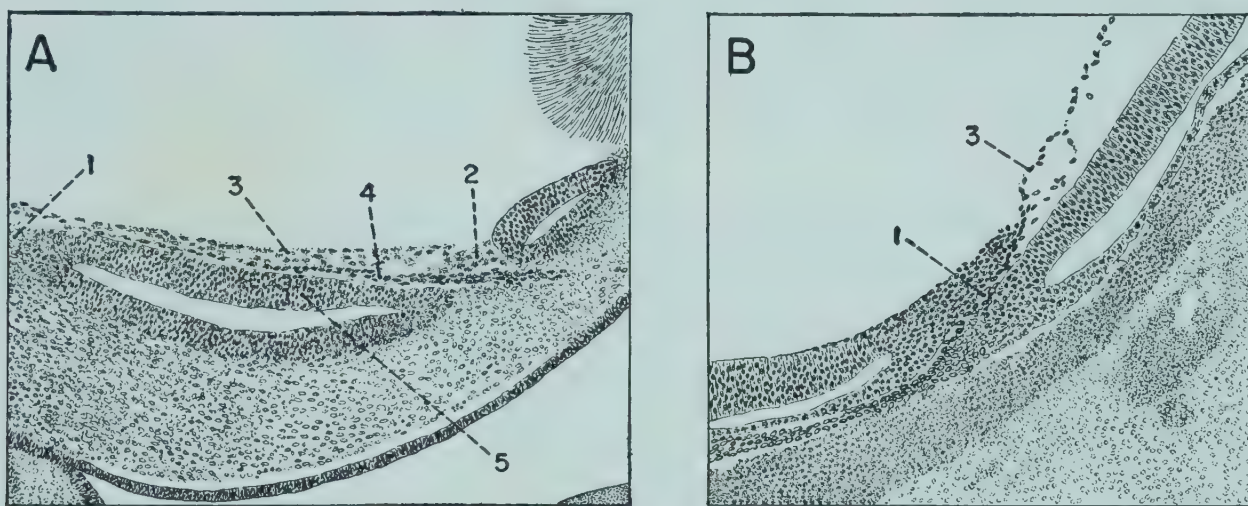


Fig. 158. Sections through the eye of the 6.5-day duck (*Anas platyrhynchos*) embryo, showing the inclusion of mesodermal elements and a blood vessel within the optic vesicle after partial closure of the chorioid fissure. (Redrawn after Lindahl and Jokl, 1922.)

A, vertical section through the ventral part of the eye, before development of the primordium of the pecten; B, cross section of the eye taken through the proximal unclosed portion of the chorioid fissure.

1, proximal opening; 2, distal opening; 3, mesoderm; 4, blood vessel; 5, ventral wall of eye.

state and serve as paths for the nerve fibers coming from the retina. As more fibers are added, the edges of the fissure are so increased in size that they grow together. The fibers from either side thus join to form a single bundle as they leave the eye (Fig. 155-B<sub>1</sub>). The primordium of the pecten develops in the trough above the fused lips of the fissure, therefore directly internal to the bundle of nerve fibers. The pecten slants toward the temporal side of the eye because of the larger size of the nasal edge of the open fissure. In their most distal portion, both the pecten and the cauda are divided in half by the mesodermal lamella, which they seem to overtake in their growth (Fig. 155-B<sub>2</sub>). The mesodermal tissue is forced out as first the pecten and then the cauda regain their undivided condition.



The early pecten is higher distally (Fig. 155; Fig. 156). In the chick embryo, it increases greatly in height and becomes thin between the sixth and the eighth day (Fig. 156). An equivalent growth occurs in the duck (*Anas platyrhynchos*) embryo by the tenth day. The superficial cells of the early pecten are epithelial-like, and the deeper cells are loosely arranged and irregular in shape.

Pigment first appears in the pecten of the chick embryo after 8 days of incubation (Husen, 1913). It is not found in the sparrow's (*Passer*

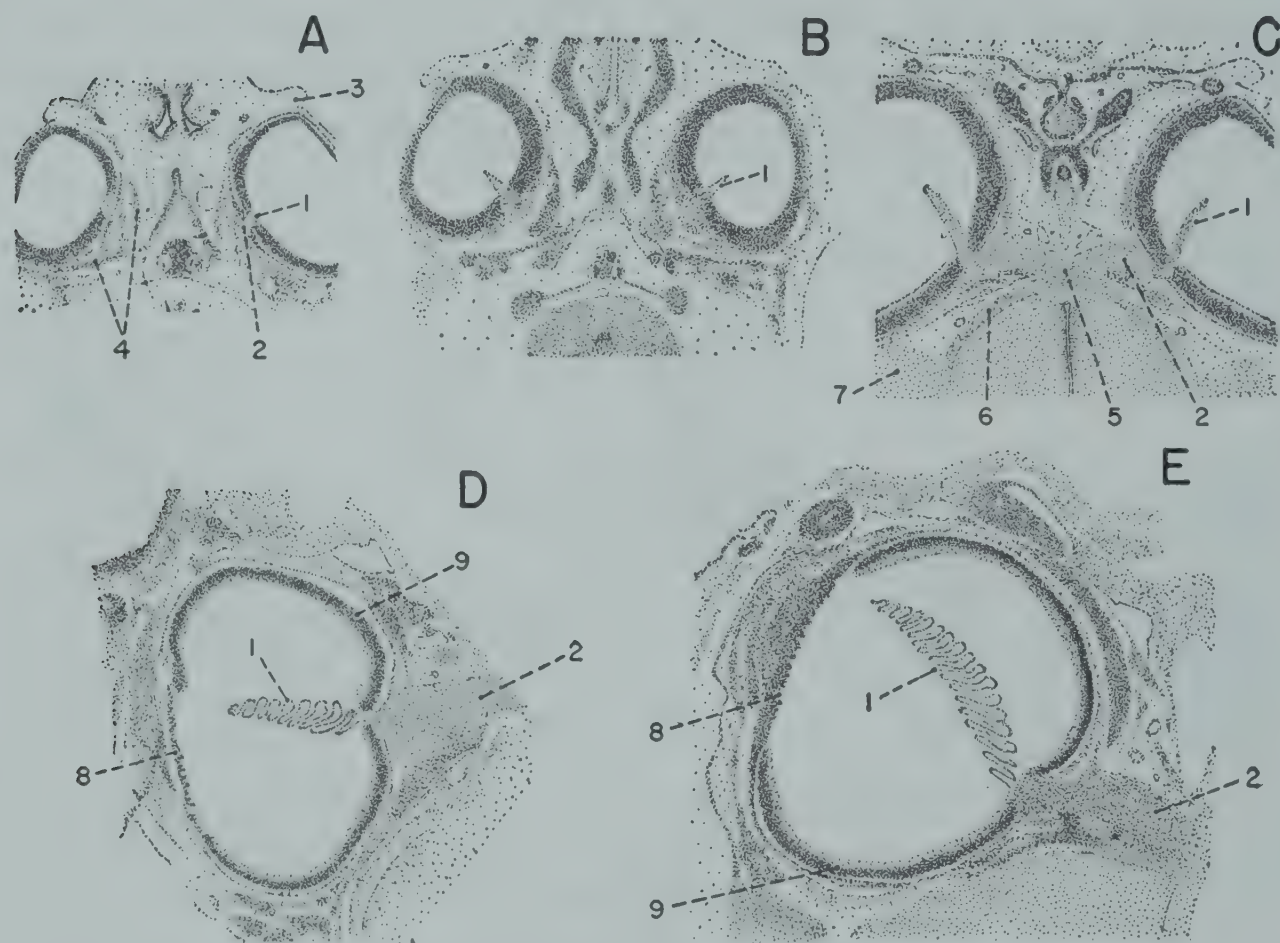


Fig. 159. The development of the pecten in the eye of the sparrow (*Passer domesticus*), shown in sections taken parallel to the equator of the eye but at a somewhat lower level. (Redrawn with modifications after Slonaker, 1921.)

A, at 7.75 days of incubation; B, at 11 days of incubation; C, at 12 days of incubation; D, 2 days after hatching (left eye); E, 4 days after hatching (left eye). All  $\times 8$ .

1, pecten; 2, optic nerve; 3, nictitating membrane; 4, eye muscles; 5, optic chiasma; 6, optic tract; 7, optic lobe of brain; 8, ciliary body; 9, retina.

*domesticus*) pecten until 4 days after hatching, and has failed to attain the adult density when the bird leaves the nest at the age of 14 or 15 days (Slonaker, 1921).

Folds begin to form in the chick embryo's pecten on the ninth or tenth incubation day (Kessler, 1877, p. 70; Froriep, 1906; Husen, 1913) about midway between the two ends. The number of folds increases rapidly and is seven on the eleventh day (Nussbaum, 1901a), fifteen on the tenth or twelfth day (Kessler, 1877, p. 70), and seventeen on the thirteenth day (Nussbaum, 1901a). In the sparrow, *Passer domesticus* (Fig. 159), folding



of the pecten does not occur until close to the hatching date, when three plications are present. The number increases to ten 2 days after hatching and reaches the full complement of twenty when the nestling is 6 days old (*Slonaker, 1921*).

In regard to the actual origin of the cells composing the primitive pecten, it seems probable that they are contributed by the retina. *Szily (1922)*, in fact, observed that cells that had apparently become detached from the inner retinal layer were lying above the line of fusion of the fissure in the 4.5 day chick embryo's eye. He regarded the presence of these elements as indicating the beginning of pecten formation. *Lindahl and Jokl (1922)* similarly noted the appearance of glia cells in the groove above the confluent nerve fibers and concluded that these cells were derived from the undifferentiated elements formerly bordering the chorioid fissure. Various other investigators have stated in more general terms their opinion that the pecten is the product of the fused lips of the fissure and hence is of retinal and ectodermal origin (*Parreidt, 1901; Bernd, 1905; Husen, 1913; Mann, 1924*).

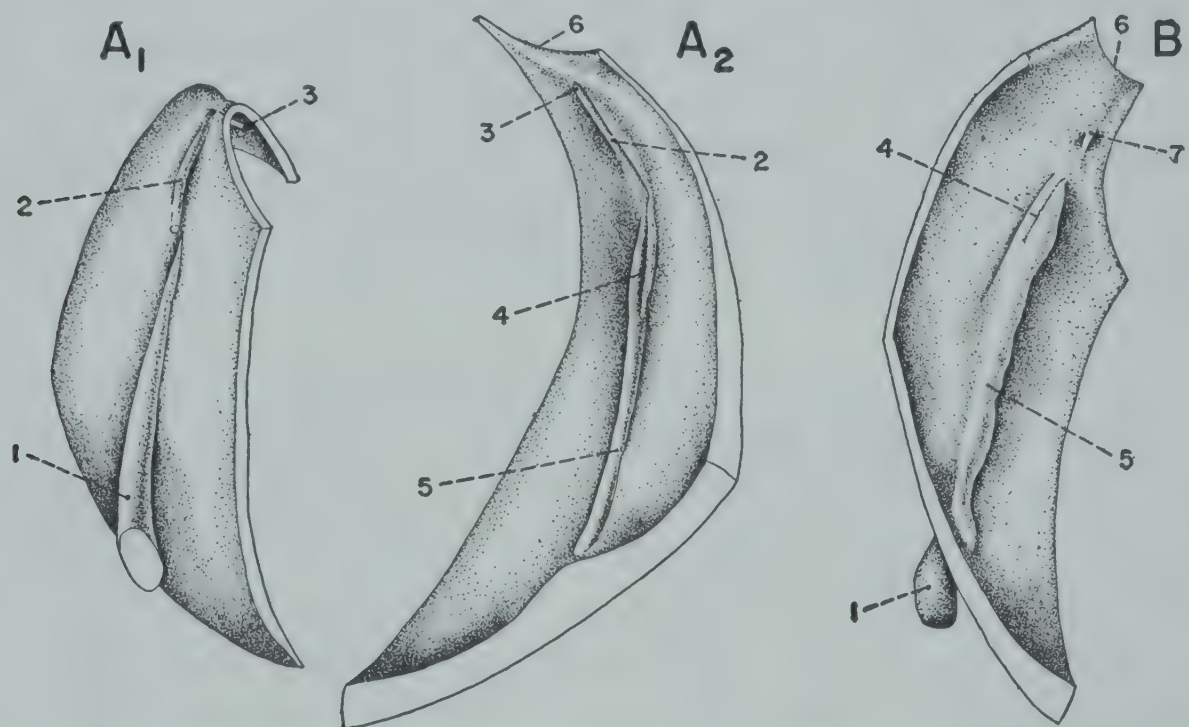
The origin and development of the pectineal circulation is more obscure. In the adult, the pecten is composed largely of capillaries. Its main blood supply is received through an artery in its base. This artery passes through the optic nerve and is fed by at least two smaller vessels (*Husen, 1913; Mann, 1924*). The principal vein is also basal in position. It communicates by small collaterals with two veins inside the sclerotic coat, one on either side of the cauda. These two veins as well as the basal vein of the pecten join a large vein which lies between the retina and the sclerotic coat along the former line of the fissure (*Mann, 1924*).

During the early period of pecten formation, the region of the chorioid fissure is served by a simple vascular loop, for the intraocular blood vessel returns proximalward over the external surface of the eye. Not all of this embryonic vascular loop persists. In the chick, the arteria cupulae opticae starts to atrophy (*Fig. 160-B*) at some time after the fifth day of incubation (*Kessler, 1877, p. 68; Husen, 1913; Lindahl and Jokl, 1922*) and has disappeared completely by the tenth day. It is possible that only its distal portion degenerates (*Husen, 1913*). An eversion of the retina within the ciliary body persists throughout adult life and marks the point where the intraocular blood vessel made its exit from the eye (*Nussbaum, 1901a; Lindahl and Jokl, 1922*).

In the description of the vascularization of the pecten given by *Mann (1924)*, it is indicated that the blood vessel visible in the base of the primordial pecten is a proximally directed branch of the intraocular blood vessel (which *Mann* regarded as homologous with the hyaloid vessel of mammals). *Szily (1922)* also found the early pectineal vessel to be continuous distally with the intraocular blood vessel, but *Lindahl and Jokl*



(1922) found no connection. Mann (1924) further observed that the proximal branch of the "hyaloid" vessel is included within the pecten at the time the anlage of the latter is formed. She also stated that the definitive basal pectineal artery is first visible on the chick's sixth incubation day as a minute capillary extending from the vascular system of the optic nerve. The pecten has meantime started to become vascularized through the proliferation of the earlier vessel, which eventually becomes the basal pectineal vein. Vascularization continues until the entire pecten comes to consist of a capillary network interspersed with and supported by a sparse pigmented tissue of neuroepithelial origin.



**Fig. 160.** The relationship between the optic nerve, the pecten, the arteria cupulae opticae, and the closing chorioid fissure, as shown in views of a portion of the ventral wall of the chick embryo's eye. (Redrawn with modifications after Lindahl and Jokl, 1922.)

A<sub>1</sub> and A<sub>2</sub>, external and internal views, respectively, of eye of 6.5-day embryo; B, internal view of eye in a slightly more advanced stage of development. All  $\times 10$ .

1, optic nerve; 2, arteria cupulae opticae; 3, distal, and 4, proximal open portion of chorioid fissure; 5, pecten; 6, pupillary margin; 7, remnant of degenerating blood vessel.

According to Husen (1913), it is the distal portion of the basal pectineal artery which is derived from the proximal portion of the intraocular blood vessel. The midportion and proximal part of the definitive vessel then appear in succession. The vein which follows the original line of the chorioid fissure under the sclerotic coat develops from the portion of the embryonic vascular loop lying outside of the optic vesicle. Mann (1924) noted that the two veins paralleling the cauda appear fairly early in incubation and represent the division of a single vessel originally seen directly external to the chorioid fissure. These two vessels again coalesce distally into a large trunk which receives the pectineal vein near the end of the cauda.



### The Iris and the Ciliary Body

The portion of the retina between the pupillary margin and the ora serrata becomes associated with tissue of mesodermal origin to form the iris, surrounding the pupil, and the ciliary body, concentric with the iris peripherally.

In the 4-day chick embryo, the margin of the optic cup is pressed against the equator of the lens, whose outer surface is everywhere in close proximity to the overlying ectoderm (*Lenhossék, 1911*). A sheet of mesenchymal tissue has grown in between the ectoderm and the outer layer of the optic vesicle. The free, invading edge of the mesenchymal sheet encircles the lens and lies immediately external to the margin of the optic cup, which, of course, is the future pupillary margin (cf. Fig. 163-A).

#### *The Iris*

In the 7- to 8-day chick embryo—or the 8- to 10-day duck (*Anas platyrhynchos*) embryo (*Lindahl, 1915*)—the outermost layers of mesenchymal tissue start to grow out farther beneath the surface epithelium covering the pupillary opening (cf. Fig. 163-C and D), to enter into the formation of the cornea. Simultaneously, the lenticular portion of the retina becomes thinner by expansion, and its margin extends somewhat farther over the lens (*Kessler, 1877, p. 96; Lindahl, 1915*). The most internal layers of the sheet of mesenchyme also grow centripetally, but their growth brings them only as far as the pupillary border. The intermediate mesenchymal tissue grows less rapidly than the inner and outer strata. As a result, the innermost layers of mesenchyme are gradually separated from the outermost layers (the corneal tissue) by a space (Fig. 161-A; cf. Fig. 163-C and D). This space is the circumferential, or peripheral, portion of the anterior chamber of the eye.

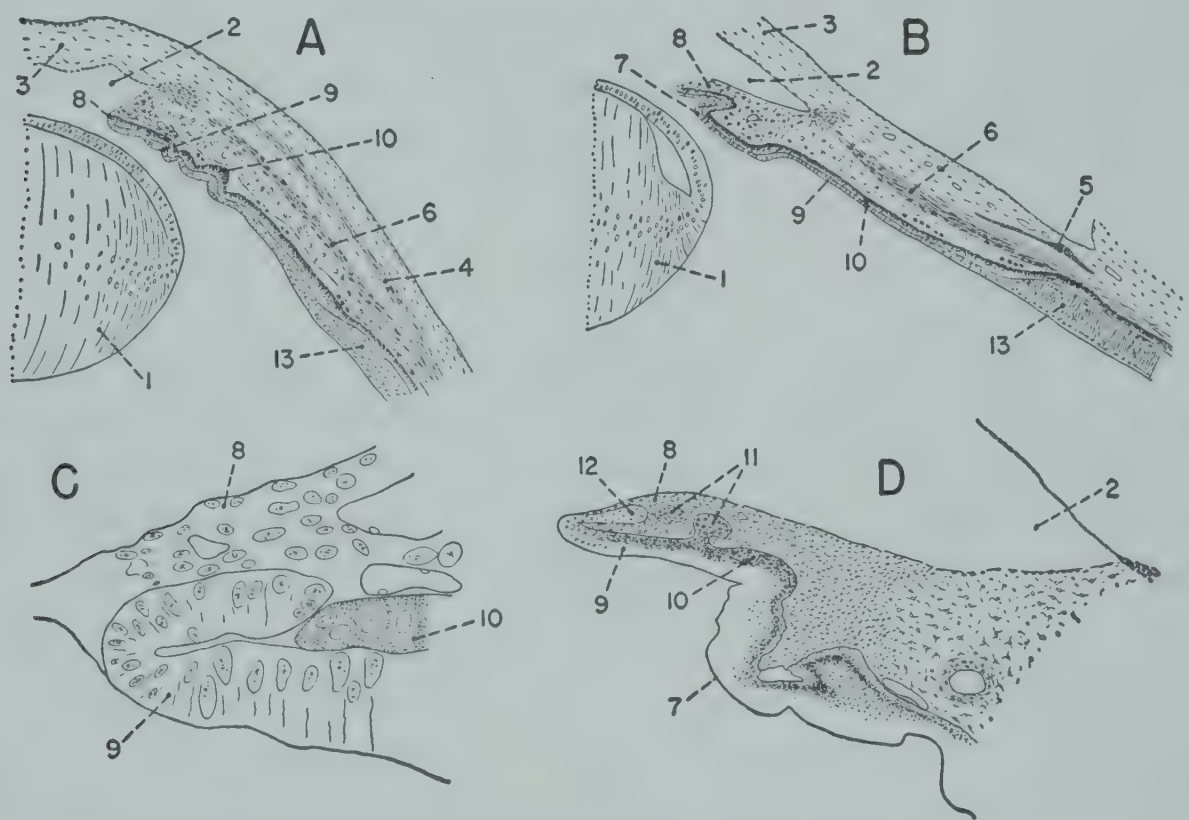
The primordial iris consists of the tissue, mesenchymatous and retinal, bounding the peripheral part of the anterior chamber posteriorly and separating it from the vitreous body. The peripheral limit of the iris becomes demarcated when the anlagen of the ciliary processes start to project toward the lens, internal to the iris (Fig. 161-B).

In the chick embryo, the sphincter muscle of the iris begins to develop on the eighth day (*Collin, 1903*) or the ninth day (*Durand, 1893a, 1893b; Lewis, 1903; Leplat, 1912*); in the sparrow (*Passer domesticus*), it first appears about at the time of hatching (*Slonaker, 1921*). The sphincter muscle is circular and occupies the external face of the iris from the pupillary to the ciliary border; it serves to contract the pupil. In birds, it is much larger than in mammals and differs further by being composed



of striated muscle; but, as in all vertebrates, it is of ectodermal origin, since it is derived from the margin of the secondary optic vesicle.

The formation of the sphincter muscle in the chick embryo is foreshadowed at the end of the seventh day of development by the appearance of a narrow, nonpigmented zone around the pupillary margin (Lewis, 1903). This zone represents an eversion of the inner retinal layer (Fig. 161-C), whose margin has grown more rapidly than the margin of the pigmented layer (Collin, 1903; Leplat, 1912). The everted portion soon enlarges, and, on the eighth day, it can be recognized as being composed



**Fig. 161.** Stages in the development of the ciliary body and the iris and its sphincter muscle in the eye of the chick embryo, as seen in sections. (Redrawn with modifications A and B, after Leplat, 1912; C and D, after Lewis, 1903.)

A, at 8 days ( $\times 30$ ); B, at 12 days ( $\times 15$ ); C, at 7 days ( $\times 400$ ); D, at 10.5 days of incubation ( $\times 100$ ).

1, lens; 2, anterior chamber of eye; 3, cornea; 4, sclerotic coat; 5, primordium of scleral bone; 6, primordium of ciliary muscle; 7, ciliary process; 8, iris; 9, inner layer of retina; 10, outer layer of retina; 11, bud from outer retinal layer; 12, bud from inner retinal layer; 13, ora serrata.

of buds of tissue overlapping the pigmented layer. The buds do not form a continuous band around the pupil until the tenth day (Lewis, 1903).

About a day after the appearance of buds derived from the inner retinal layer, additional buds start to arise from the external surface of the pigmented layer of the iris anlage (Fig. 161-D) and to migrate into the overlying mesenchymatous stroma (Lewis, 1903; Leplat, 1912). Their development progresses outward from the margin of the pupil. Their number increases until the eleventh day of incubation and then diminishes. After penetrating into the mesenchymal tissue of the iris, the pigmented



cells composing the buds are transformed into myoblasts which lose their pigment after the thirteenth day. At this time, striated muscle fibrils appear in the myoblasts (*Leplat, 1912*), and the sphincter muscle extends to the inner ciliary border (*Durand, 1893b*).

Beneath the sphincter muscle there are radial striated muscles in the iris of many birds. In some species, such as the chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*), and goose (*Anser anser*), the radial muscles are arranged in two layers, an internal layer of fine, truly radial fibers and an intermediate layer of thick, obliquely radial fibers; in other species (pheasant, *Phasianus colchicus*, guinea hen, *Numida meleagris*) only the oblique layer underlies the sphincter muscle; in the pigeon (*Columba livia*), there are almost no radial fibers at all. Both layers of radial muscles are inserted peripherally into the connective tissue of the ciliary body (*Durand, 1893b; Leplat, 1912*). Their function has generally been taken as dilative, but it has also been proposed (*Leplat, 1912*) that they play a part in accommodation by exerting traction on the ciliary bodies, which then compress the lens (the ciliary processes being in contact with the lens, in birds). *Slonaker (1920)*, however, denies the possibility that the firm avian lens can be made to change its shape by the action of the ciliary muscles.

The radial muscle fibers appear later than the circular. In the chick, the obliquely radial fibers are first recognizable on the thirteenth day of incubation and the innermost radial fibers on the nineteenth (*Durand, 1893b*). Their development proceeds centripetally. All the radiating muscle fibers of the iris take their origin from the same elements as the circular (*Leplat, 1912*).

### *The Ciliary Body*

The ciliary body is of more complex structure than the iris. Its folds, which may number from 85 to 150 in different species of adult birds, radiate from the lens along meridians of the eye. The ciliary processes, or central terminations of the folds, project beneath the periphery of the iris and contact the lens (cf. Fig. 162-E). The stroma of the processes is elastic tissue of mesodermal origin; this tissue, which lies immediately external to the pigment layer of the ciliary portion of the retina, extends to the periphery of the ciliary body. Between the elastic or conjunctivo-vascular layer of the ciliary body and the sclerotic coat of the eye lies the ciliary muscle (cf. Fig. 161-A and B), composed of three divisions and extending obliquely from the level of the ora serrata to the periphery of the anterior chamber. The angle between the ciliary muscle and the layer of elastic tissue is filled by the pectineal ligament, a loose network of fine, elastic fibers radiating from a point at the periphery of the cornea to the elastic layer of the ciliary body and to the peripheral part of the



iris. The pectineal ligament preserves the proper spatial relationship between the iris and the ciliary body during active accommodation of the eye.

The first ciliary processes to form in the chick embryo have been said (Kessler, 1877, p. 96) to appear on the ninth or tenth day of incubation. They have also been seen, however, during the course of the sixth day (Nussbaum, 1901a) and at the end of the eighth day (Lewis, 1903). Their development apparently is initiated on the temporal side of the eye, where the ciliary body will eventually be widest (Nussbaum, 1901a). The early processes consist of lensward projections of mesenchyme covered on the internal surface by the ciliary retina (cf. Fig. 161-B). About ninety are present in the chick of 16 to 17 days' incubation (Nussbaum, 1901a).

Where the retina bends around to continue over the iris, buds of pigmented cells from the outer retinal layer migrate into the mesenchymal stroma of the ciliary processes. During the tenth day, this layer of the retina starts to give off pigmented buds into the more peripheral stroma of the ciliary body (Lewis, 1903; Leplat, 1912). After the thirteenth day, mesodermal cells in this region start to acquire pigment (Leplat, 1912).

The primordial ciliary muscles make their appearance at about 8 days of incubation as a band of myoblasts extending beneath the sclerotic coat from the ora serrata almost as far as the periphery of the cornea (Fig. 161-A and B). On the eleventh day, the first indication of transverse striation is seen in the chick (Leplat, 1912); in the sparrow (*Passer domesticus*), striation of the ciliary muscle begins 2 days after hatching and is not completed until the bird leaves the nest (Slonaker, 1921). The three divisions of the ciliary muscle have differentiated on the chick's sixteenth day of development, and little further change occurs (Leplat, 1912.)

The pectineal ligament develops through differentiation *in situ* of primitive mesenchyme. On the twelfth day of incubation, the mesenchyme in the region of the future ligament is much looser than elsewhere and the cells are united by cytoplasmic prolongations which are beginning to orient themselves in the direction later to be followed by the fibers of the ligament. A day and half later, fine elastic fibers have formed within the cytoplasm, and, on the sixteenth day, the ligament is present, though more tenuous than in the adult bird (Angelucci, 1881; Leplat, 1912).

An elastic-walled venous sinus known as the canal of Schlemm appears external to the pectineal ligament on the thirteenth or fourteenth day. It encircles the anterior chamber and is part of a venous plexus (Angelucci, 1881; Leplat, 1912).

According to Slonaker (1920), the ciliary muscles effect accommodation in the bird's eye by acting on the sclerotic coat to change the equatorial diameter of the eyeball. Contraction of the ciliary muscles would



thus reduce this diameter, increase intraocular pressure, and push the lens and the anterior portion of the eye forward for near vision. This concept likens visual accommodation in the bird to the focusing of a camera. Other and older theories (*Exner, 1882; Beer, 1893; Hess, 1913*) proposed that accommodation is achieved, at least in part, by the action of the ciliary muscle on the cornea, which indirectly alters the tension of the pectineal ligament and changes the shape of the lens.

### **The Cornea, the Anterior Chamber, the Vitreous Body, and the Lens Ligament**

The epithelium of the cornea (the conjunctiva) is derived from the primitive surface ectoderm of the embryo, but mesenchyma contributes largely to its subepithelial portion. The development of the deeper layers of the cornea is initiated by the appearance of a delicate fibrous membrane which forms within the early anterior chamber of the eye and which is part of a fibrous complex giving rise also to the vitreous body.

The vitreous or posterior chamber starts to appear early in the chick's third day of incubation as a space between the fundus of the invaginating optic cup and the lens vesicle, which has not yet closed completely (cf. Fig. 148-D). From the beginning, the vitreous chamber contains fine fibers (*Knape, 1909; Laguesse, 1926; Nordmann, 1938*), the early constituents of the vitreous body or humor. These fibers appear to be continuous with a slightly denser fibrous membrane loosely applied to the medial surface of the lens vesicle (cf. Fig. 149-F), that is, to the surface which is not in contact with the superficial ectoderm (*Nordmann, 1938*). It has been suggested that this membrane represents the basal membrane of the lens placode, pushed inward as the lens vesicle invaginates (*Laguesse, 1926; Nordmann, 1938*). At the point where it passes between the lens vesicle and the margin of the optic cup, the membrane joins additional noncellular material which apparently proceeds from the very narrow space intervening between ectoderm and both lens vesicle and optic cup (cf. Fig. 149-F).

During the chick's fourth day of incubation, the space between the closed lens vesicle and the ectoderm overlying it—which now has a more pronounced curvature than elsewhere—becomes wider and is recognizable as the early anterior chamber of the eye (cf. Fig. 163-A). At first, the anterior chamber is filled with a rather homogeneous substance (*Watzka, 1935*), which very soon undergoes a condensation into the fibers of the transitory “anterior vitreous body” of early authors. This condensation leads, in particular, to the formation of another membrane lying close beneath the surface epithelium (cf. Fig. 163-A). A loose network of fibers situated at the margin of the optic cup connects this membrane with the membranous and fibrous elements of the vitreous body and with the



invading mesenchyme, which is now approaching the pupillary border from all sides. It has been said that the loose fibrous network, together with a small amount of fibrous material scattered throughout the anterior chamber, suddenly condenses at 6 days of incubation into a membrane covering the anterior surface of the lens.

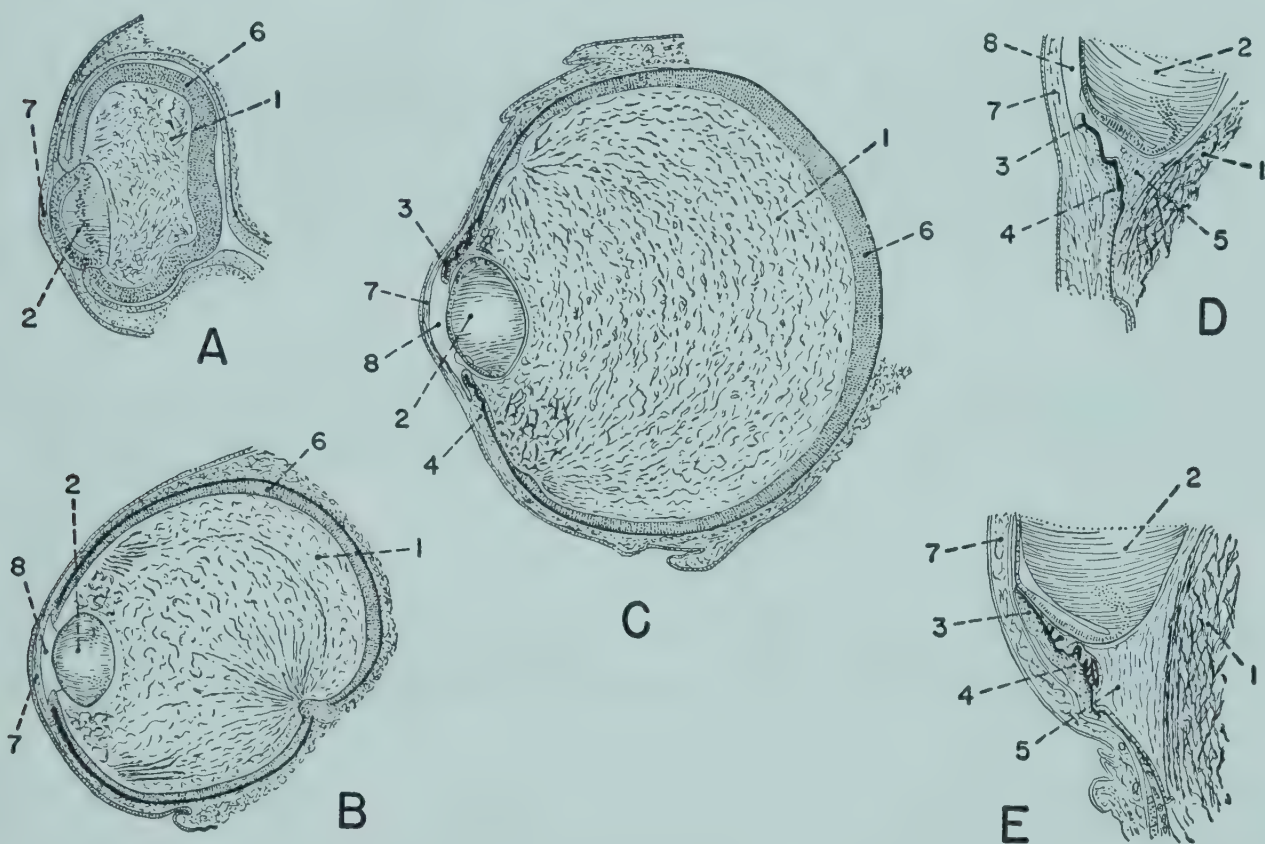
There has been considerable disagreement regarding the origin of the fibrous material which figures so prominently in the development of the eye. It has been suggested that the fibers are the direct product of the cells of the epithelium, lens, and retina (*Lenhossék*, 1903, 1911; *Szily*, 1904, 1908; *Laguesse*, 1926), and that, although they are of ectodermal origin, they resemble the material (mesostroma, plasmodesma, or intercellular bridges) elaborated in common by all types of embryonic cells (*Szily*, 1904, 1908; *Laguesse*, 1926). *Angelucci* (1881) spoke of the vitreous body as a derivative of the mesodermal hyaloplasm or interstitial substance. *Watzka* (1935) observed that the vitreous body differed in appearance from the material in the anterior chamber, and was inclined to consider the latter as the product of degenerated mesenchymal cells. To *Kessler* (1877, *p.* 34), the vitreous body was a simple transudate from the blood vessels in the chorioid fissure.

The later development of the vitreous body has been described by *Lenhossék* (1911). This part of the 4-day chick embryo's eye consists of a fibrous network which shows no particular arrangement except for the fact that a few fibers radiate from either side of the inner wall of the optic vesicle at a point near the lens (Fig. 162-A). These fibers do not seem to emanate from the future retinal cells. On the seventh incubation day, however, a definite pattern is apparent. Fibers may now be seen radiating in all directions from the primordium of the pecten (Fig. 162-B). The most peripheral of these fibers follow the curving contour of the optic vesicle some distance from its wall and are continuous with those which radiate, as before, from the anterior wall. The points of origin of the latter fibers have shifted away from the lens, because of the growth of the lenticular portion of the retina, and are now at the ora serrata. The arrangement on the tenth day is much the same, except that some of the fibers radiating from the ora serrata now extend toward the lens and some pass straight behind it (Fig. 162-C). On the fourteenth day, the early arrangement of the fibers in the anterior part of the vitreous body has disappeared. The fibers behind the lens are thicker and are condensing and fusing together; they are now circumferential in direction. The fine fibers originating at the ora serrata pursue a curving course through the small space between the ciliary body and the lateral surface of the lens and no longer extend into the vitreous body (Fig. 162-D). It is clear that they represent the developing lens ligament, or zonula ciliaris. It should be mentioned, however, that *Angelucci* (1881) indicated the



presence of lens ligament fibers in their definitive position in an illustration representing a section of an 8- to 9-day chick embryo's eye. Eventually, the fibers of the zonula become fused with the lens capsule and with the limiting membrane of the ciliary body (Fig. 162-E).

The cornea is built up from within outward, toward the surface epithelium, which will become part of it. The first step in the development of corneal tissue is preceded, on the chick's fourth incubation day, by the formation of a fibrous membrane beneath the epithelium of the anterior chamber (Fig. 163-A), as previously described. Early in the fifth day, when the mesenchyme has reached the margin of the optic cup, single mesen-



**Fig. 162.** The development of the vitreous humor and the lens ligament in the chick embryo's eye. (Redrawn with modifications after Lenhossék, 1911.)

A, at 4 days ( $\times 36$ ); B, at 7 days ( $\times 12$ ); C, at 10 days ( $\times 12$ ); D, at 14 days ( $\times 18$ ); E, at 16 days of incubation ( $\times 18$ ).

1, vitreous humor; 2, lens; 3, iris; 4, ciliary body; 5, fibers of lens ligament; 6, retina; 7, cornea; 8, anterior chamber.

chymal cells start to migrate in from all sides along the inner surface of the membrane (Kessler, 1877, *p.* 84; Knape, 1909; Laguesse, 1926; Watzka, 1935). Twenty-four hours later, they have formed a continuous, single-layered stratum of flattened cells, the corneal endothelium (Fig. 163-C). Based on investigations of chick embryos between the fourth and eighth incubation days, Coulombre (1956*b*) suggests that tangential force, arising from intraocular pressure, is one factor in the normal increase in diameter of the developing cornea.

While the endothelial cells are still migrating, a new fibrous layer appears external to them, and the first fibrous membrane seems to disappear



(Watzka, 1935). The new layer consists of several strata of undulating collagenous fibers (cf. Fig. 163-B). It extends peripherally as far as the sclera (Lenhossék, 1911; Watzka, 1935), and increases continually in thickness up to the end of the sixth day (cf. Fig. 163-C). It may be a product of either the epithelial cells (Kessler, 1877, *p.* 88; Laguesse, 1926) or the endothelial cells (Watzka, 1935).

As soon as the corneal endothelium is formed, the development of the substantia propria of the cornea begins. This step is interrelated with the evolution of the iris and ciliary body and takes place during the seventh day (Kessler, 1877, *p.* 84; Laguesse, 1926; Watzka, 1935). The mesenchyme

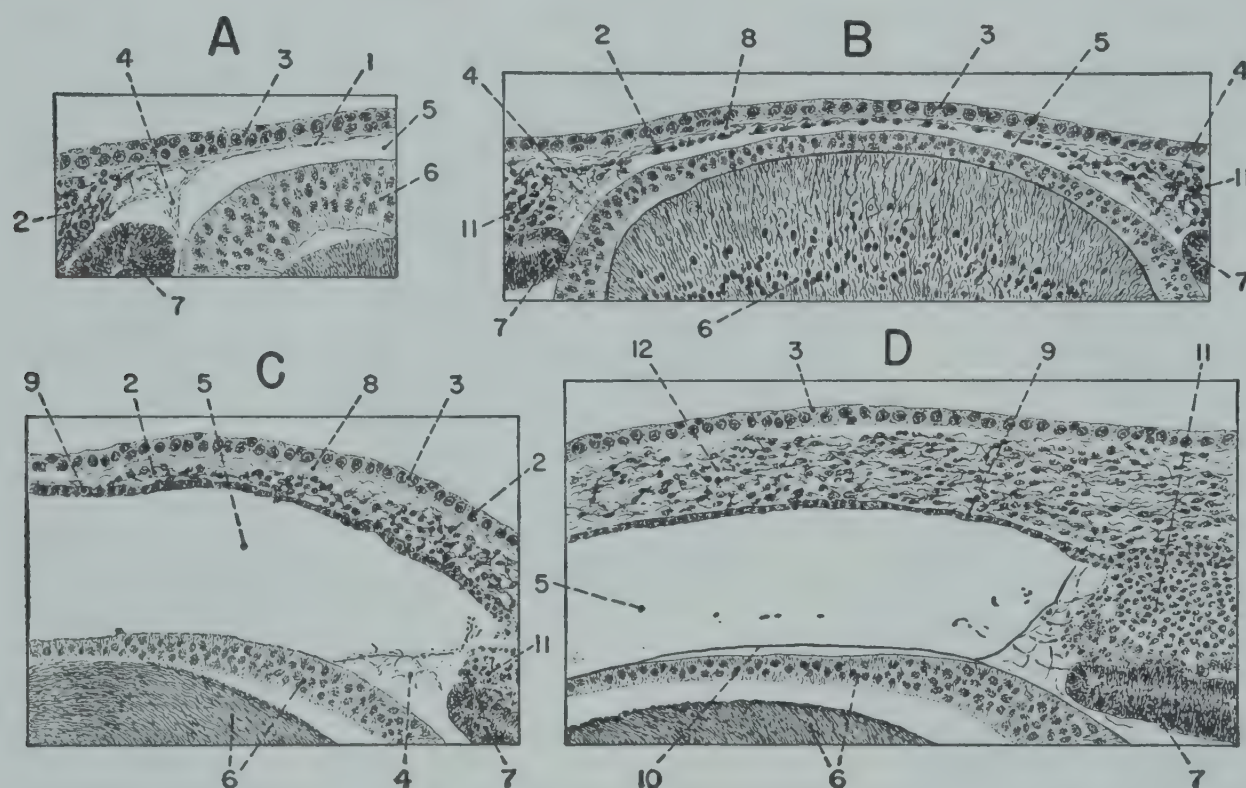


Fig. 163. Successive stages in the development of the cornea in the chick embryo's eye. (Redrawn with modifications after Watzka, 1935.)

A, at 4 days; B, at 5 days; C, at 6 days; D, at 8 days of incubation. All  $\times 100$ .

1, fibrous membrane; 2, mesenchymal cells; 3, corneal epithelium; 4, fibrous network; 5, anterior chamber; 6, lens; 7, margin of optic cup; 8, layer of collagenous fibers, primordium of Bowman's membrane; 9, corneal endothelium; 10, anterior lens capsule; 11, mesenchyme of future iris; 12, substantia propria of cornea.

overlying the margins of the optic cup (or retina) becomes divided into two parts, an outer and an inner. The latter remains associated with the retina and will soon contribute to the further development of the iris and the ciliary body. The outer portion, however, continues to grow centripetally, invading the newly formed fibrous layer of the cornea (cf. Fig. 163-C).

This division of the mesenchyme affects the shape of the anterior chamber, which, at the beginning of the seventh day, has increased considerably in depth. Its periphery, in cross section, forms a curve with convexity facing outward. Now, as the mesenchyme grows into the iris and the cornea, this curve is gradually transformed into an acute angle.



Mesenchymal cells invade the middle zone of the fibrous layer of the cornea (Kessler, 1877, *p.* 85). The outer cell-free zone, however, is much wider than the inner (Watzka, 1935). As the cellular zone increases in thickness (Fig. 163-D), the outer fibrous layer becomes continually denser by compression and is recognizable on the eleventh day as the primordium of Bowman's membrane (which lies immediately beneath the epithelium). The inner fibrous layer has been said to give rise to Descemet's membrane (Kessler, 1877, *p.* 85; Laguesse, 1926), overlying the endothelium. It is also said that the inner fibrous layer disappears and that Descemet's membrane is formed between the thirteenth and fifteenth day as a new product of the corneal endothelium (Watzka, 1935).

The outer epithelium of the cornea is the last layer to complete its development, although it is the first to be present. It is originally represented by the two cell layers composing the superficial ectoderm. The outer layer consists of horizontal cells which change only by increasing in number and in density of arrangement. The inner layer of cells becomes transformed into cylindrical epithelium. Toward the end of incubation a third layer of round cells appears between the first two (Kessler, 1877, *p.* 85).

### The Chorioid and Sclerotic Coats

The chorioid and sclerotic coats are continuous with the ciliary body and the cornea, respectively, and occupy the posterior two thirds of the eyeball. The chorioid coat is a pigmented, vascular layer immediately external to the retina. The sclerotic coat, external to the chorioid, is cartilaginous and fibrous and, in birds, terminates marginally in a ring of bony plates. Both the chorioid and the sclera arise from the mesenchyme which invests the optic cup during embryonic development and which contributes also to the formation of the ciliary body and the cornea. The differentiation of the chorioid and the sclerotic coats begins at the posterior pole of the optic vesicle and proceeds toward its margin.

#### *The Chorioid Coat*

The development of the chorioid coat has been observed in the chick by Leplat (1912) and in the sparrow (*Passer domesticus*) by Slonaker (1921). The earliest differentiation of the chorioid consists of a condensation of the mesenchyme contiguous to the outer layer of the optic cup. This condensation of tissue is apparent in the 2-day sparrow embryo and the 5-day chick embryo. The innermost layer of mesenchyme is composed of elongated or spindle-shaped cells arranged concentrically around the optic cup. These cells are surrounded by a layer of capillaries, external



to which are additional spindle-shaped cells representing early elements of the sclera.

The next stage in the development of the chorioid consists chiefly of an increase in the size and conspicuousness of the capillary network. The capillary layer is not sharply demarcated from the sclera until the latter becomes cartilaginous on the eighth to ninth day of incubation of both chick and sparrow (*Passer domesticus*). In a culture of fibroblasts from the chorioid and sclerotic of an 11-day chick embryo Phillips (1952) found that cell density rises steadily from the periphery toward the center of the colony. Maximum mitotic index is in the region of moderate cell density with the relative proportions of the different phases of mitosis the same in all parts of the colony.

In the chick, a layer of larger vessels appears external to the capillary layer on the thirteenth to fourteenth day of development. About a day later, a connective tissue (adventitial) sheath appears around the endothelial walls of the larger vessels. On the sixteenth day, elastic fibers can be distinguished in the adventitia of the large vessels and in the collagenous connective tissue between the vessels. The internal limiting membrane of the chorioid (the membrane of Bruch), composed of elastic tissue, becomes clearly recognizable on the eighteenth day.

Pigmentation of the connective tissue of the chorioid begins in the chick embryo on the eighth day of incubation. It proceeds slowly until the fourteenth day, then increases rapidly until it reaches its maximum on the eighteenth day. In the sparrow (*Passer domesticus*), however, the chorioid remains unpigmented until a day or two after hatching. In the opinion of Busacca (1913), the pigment of the chorioid is derived from mitochondria.

### *The Sclerotic Coat*

The development of the sclerotic coat begins simultaneously with that of the chorioid coat. In the eye of the 4-day sparrow (*Passer domesticus*) embryo, the future sclera is sharply marked off from the mesoderm surrounding it externally. In the chick or sparrow (*Passer domesticus*) embryo, true scleral cartilage is first apparent on the eighth or ninth day of incubation (Leplat, 1912; Slonaker, 1921; Weiss and Amprino, 1940). At this time it forms a curved plate three or four cell layers deep around the chorioid and is delimited internally and externally by spindle cells which will become the internal and external perichondrium, respectively.

The thickness of the embryonic scleral cartilage is probably influenced by the tension which the growth and expansion of the eye produces in the surrounding mesenchyme. Abnormally thick scleral cartilage forms around eyes stunted by removal of the vitreous humor on the fourth day (Weiss and Amprino, 1940).



The future course of differentiation to be taken by the prescleral mesenchyme is apparently determined on the fourth day, when this tissue first exhibits the capacity for self-differentiation *in vitro*. Its determination is perhaps due to some inductive action by the optic cup (Weiss and Amprino, 1940).

The early scleral bones (cf. Fig. 161-B) can be recognized on the ninth day of incubation in both the chick and the sparrow (*Passer domesticus*). Their appearance, however, is preceded by the formation of a ring of fourteen papillae in the conjunctival epithelium, as shown in Fig. 164

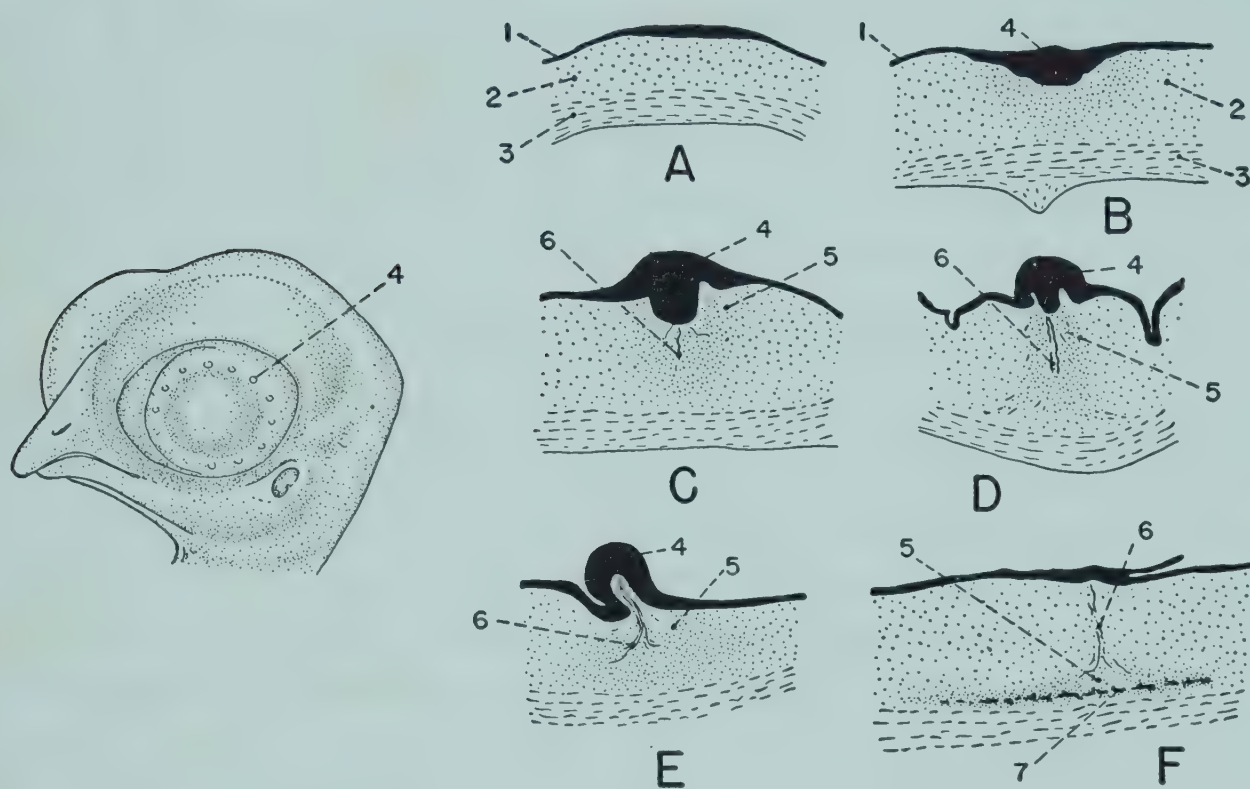


Fig. 164. Diagrams showing successive stages in the development of the scleral bones in the chick embryo's eye, and the role played in their development by epidermal papillae. (Redrawn with modifications after Murray, 1943.)

At left: A view of the 7-day embryo's head, showing the location of the epidermal papillae. (Redrawn with modifications after Keibel and Abraham, 1900.)

A, at 7 days; B and C, at 7 to 8 days; D and E, at 8 to 9 days; F, at 9 to 10 days of incubation. All  $\times 55$ .

1, epidermis; 2, mesenchyme; 3, fibrous sclera; 4, epidermal papilla; 5, mesenchymal condensation; 6, collagen fibers; 7, precursors of bony plate.

(Nussbaum, 1901b; Dabelow, 1926; Murray, 1943). In the chick, these papillae are rather flat on the seventh day (Fig. 164-A). By the eighth day, however, they have formed slight projections above the surface and have also grown downward into the mesenchyme (Fig. 164-B and C), which has undergone a slight condensation beneath each papilla. The papillae then begin to degenerate from the interior outward (Fig. 164-D and E). Simultaneously, the mesodermal condensations start to migrate downward and to flatten out (Fig. 164-D, E, and F), and collagen fibers appear. These extend out from the basement membrane of the papilla



among the mesenchyme cells, some of which are degenerating. The cells of the flattened mesenchymal condensations start to transform into osteoblasts (Fig. 164-F), and collagen fibrils appear among them as a matrix. These fibrils become continuous with the collagen fibers proceeding from the degenerating papillae (which completely disappear by the twelfth day of incubation). Later, the scleral bones increase in thickness by additions to their internal surface, and they grow in extent in every direction except centrifugally. An internal and an external fibrous periosteum develops; the latter is continuous with the external perichondrium of the scleral cartilage, and the former merges with a fibrous continuation of the sclerotic coat overlying the ciliary muscles (Murray, 1943).

Slonaker (1920) has suggested that the scleral bones serve a reinforcing function and prevent the collapse of the eye when the ciliary muscles contract.

### The Extrinsic Eye Muscles

In birds, as in other animals, the eye is moved in its orbit by the four rectus and the two oblique muscles. Two additional muscles, the pyramidalis and the quadratus nictitans, are found on the medial surface of the bird's eyeball. These are the muscles controlling the nictitating membrane, which is translucent and moves over the eye very frequently and rapidly (Slonaker, 1918). Three different cranial nerves supply the eye muscles: the oculomotor nerve serves the inferior oblique muscle and all the rectus muscles except the lateral (external); the trochlear nerve innervates the superior oblique muscle; and the abducens nerve supplies the pyramidalis, quadratus nictitans, and lateral rectus muscles.

The extrinsic eye muscles are derived from mesodermal condensations which appear posterior to the eye and grow toward their definitive positions (Rex, 1897, 1901, 1905, 1924; Edgeworth, 1907; Matys, 1908; Slonaker, 1921; Adelman, 1927), but there is some disagreement as to the original location and subsequent development of several of the muscle primordia. The account given by Adelman (1927) follows below.

The common primordium of the muscles supplied by the abducens nerve appears in the chick of 24 or 25 somites dorsal to the mandibular arch and anterior to the first visceral pouch. At the 29-somite stage, a second condensation is found dorsal to the hyoid arch. The more anterior portion of this condensation is the anlage of the superior oblique muscle; the remainder eventually disappears, although it possibly contributes both to the lateral rectus muscle and to the sclera. At the 33-somite stage, a condensation representing the primordium of the superior rectus muscle forms medial to the superior oblique anlage. At the 40-somite stage, the



inferior oblique anlage can be identified caudoventral to that of the superior rectus. The inferior and medial rectus muscles arise from a common primordium visible at the 47-somite stage dorsal (caudal) and medial to the optic stalk.

As development proceeds, these condensations grow in a general anterolateral direction, toward the expanding eyeball. Thus, after 87 hours of incubation, the mesodermal tissue mass comprising the oculomotor-supplied muscle anlagen has surrounded the medial side of the optic cup, and the condensation bordered cranially by the superior oblique primordium is applied to the dorsocaudal surface of the eye. More caudally, the common primordium of the abducens-supplied muscles also swings anterolaterally, and, at 120 hours, its long axis is directed mediolaterally.

The anlage of the superior rectus muscle, the first of the oculomotor-supplied group to appear, continues its lateralward growth until, at 120 hours, it comes into contact with the eye and fuses with the sclera. This muscle is innervated at about 102 hours.

The inferior oblique muscle anlage, the second of this group to form, grows laterally along the chorioid fissure and, at 102 hours, lies ahead of the fissure with its long axis mediolateral. Its medial end then shifts forward in the direction of its point of attachment on the nasal septum, which it reaches at about 5.5 days; its lateral end then effects its insertion in the sclera. The oculomotor nerve innervates this muscle at 72 to 75 hours (40 to 47 somites).

The anterolateral shifting of the common anlage of the inferior and medial recti brings this cell mass immediately dorsal (posterior) to the optic stalk at 87 hours, with the more lateral portion in contact with the eye just medial to the chorioid fissure. At 120 hours, this lateral portion has grown farther forward under the optic nerve and is separating to form the medial (internal) rectus. Subsequently, it spreads out over the medial surface of the eyeball and at 5.5 days is broad and thin. The medial portion of the common anlage grows ventrolaterally to attain the position of the inferior rectus muscle. It becomes continuous with connective tissue extending along the line of the chorioid fissure. Both muscles are innervated by the oculomotor nerve at 120 hours.

The superior oblique primordium grows shorter and thicker, and, at 102 hours, its medial end has shifted cephalad of its lateral end. The muscle continues to swing mediocephalad until its medial end attains its point of attachment on the nasal side of the orbit on the seventh day. Fibers of the trochlear nerve reach this muscle at 120 hours.

At about 120 hours, the already innervated anlage of the abducens-supplied musculature begins to divide into two distinct portions as the



common primordium of the pyramidalis and quadratus nictitans muscles starts to separate from the inner surface of the medial portion of this cellular condensation. At 5.5 days, the lateral portion of the original mass is recognizable as the lateral rectus. It has shifted still farther laterally and is broad and thin at the point near the equator of the eye where it is inserted into the sclera. At this time, the quadratus and pyramidalis are still joined and form a crescent-shaped mass, distinct from the lateral rectus. A day later, the pyramidalis muscle forms a prominent ventral outgrowth from the common mass, which surrounds the posterior side of the optic nerve. At 7 days, only a few fibers join the quadratus nictitans and the pyramidalis. The latter has now grown so that it lies nasal to the optic nerve, and its tendon is beginning to form. At 9.75 days, the two muscles are seen in their definitive relationship, with a thin tendon proceeding from the apex of the pyramidalis upward over the optic nerve, through a loop at the lower border of the quadratus nictitans, and thence down and around to the front of the eye.

Slonaker (1921) has noted that striations do not appear in the extrinsic eye muscles of the sparrow (*Passer domesticus*) until 2 days after hatching but are clearly present on the sixth day, when the eyes first open.

The development of the eye muscles appears to depend upon the presence of an eye of normal size growing at the normal rate. When the eye is reduced to a small rudiment as the result of injury, the muscles differentiate histologically but remain in the form of small groups of cells variously oriented. It is possible that the tension exerted by the growing ocular bulb on the surrounding tissues influences the orientation of the cells of these tissues (Amprino, 1948). Coulombre (1956a) suggests that intraocular pressure, in addition to affecting increase in eye size during embryonic development of the chick (see Appendix, Table V) operates as an integrating factor underlying the temporal correlations in the growth rates of the various component tissues of the eye.

### The Eyelid Muscles

The superior palpebral muscle, which elevates the upper eyelid, has been described as developing from the rectus superior muscle (Edgeworth, 1907). This genetic relationship was denied by Adelman (1927), who suggested that the muscle may not develop until after the chick's twelfth incubation day. This author traced the origin of the inferior palpebral muscle (which depresses the lower lid) from the branchial musculature of the mandibular arch. The muscle anlage is first visible at 120 hours as the dorsal portion of a spur projecting forward from the mandibular arch condensation. The palpebral muscles of the sparrow (*Passer domesticus*) become striated at the same time as the eyeball muscles (Slonaker, 1921).



## THE NOSE

In birds, the olfactory sense is not especially well developed. As in all air-breathing animals, the olfactory organ receives its stimuli by means of air inspired through the nasal cavity, which is the most external of the respiratory passages. The nasal cavity is divided into two lateral halves (the nasal fossae) by the nasal septum and is situated partly in the bird's upper beak and partly in the facial region anterior to the eyes. The cavity is of greater height proximally than distally. The narrow distal portion is known as the vestibule; the proximal portion is the nasal cavity proper. The external openings of the nasal fossae, known as the external nares, vary in shape and position in different species of birds. They may be round, oval, or elongated and are located anywhere from the base of the beak to the tip. The internal nasal apertures (the internal nares or choanae) are found directly above the median fissure of the palate. Projecting into each nasal fossa from its dorsal or lateral wall are the membrane-covered, cartilage-supported turbinals or conchae. The vestibular concha is a thin, curved plate arising from the dorsolateral wall; it is peculiar to birds but is not present in all avian species. In the nasal cavity proper, there are always two conchae. The more anterior of these, the inferior concha, is a thin plate rolled upon itself. The more posterior is merely a protuberance, with no free edge, found on the lateral wall of each nasal fossa, near the roof; it is known as the superior concha. Olfactory epithelium is confined to the superior concha and the roof of the nasal cavity. The homologies of the conchae are not clear. The superior concha has been considered the equivalent of the nasoturbinal (*Cohn, 1903*) and the ethmoturbinal (*Dieulafé, 1905*) of mammals and has been homologized with both the middle turbinal (or concha nasalis) (*Beer and Barrington, 1934*) and the posterior concha of the crocodile (*Gegenbaur, 1873; Born, 1879; Dieulafé, 1905*). The avian inferior concha, most often likened to the maxilloturbinal of mammals (*Cohn, 1903; Dieulafé, 1905; Beer and Barrington, 1934*), has also been homologized with the concha nasalis of the crocodile (*Gegenbauer, 1873; Born, 1879*).

The olfactory epithelium and the olfactory nerves arise from small bilateral areas of cranial ectoderm which become thickened into placodes and then invaginate. The remainder of the nasal cavity is produced by the outgrowth of the surrounding mesodermal tissue around the invaginations. The so-called respiratory epithelium lining the respiratory portion of the nasal cavity is derived from the ectoderm originally adjacent to the olfactory placodes.

It appears that, in the chick, the potential nose-forming elements are already localized in bilateral areas by the time they acquire the capacity



for independent differentiation in grafts; furthermore, the nose does not exhibit this capacity until much later than the other sense organs, and the different parts of the nose are determined not simultaneously but separately and progressively. Thus histogenesis of vestibular and olfactory epithelium occurs in grafts from 5- and 9-somite embryos, respectively, and the ability to undergo organogenesis in grafts is developed in succession by the three conchae at stages of 12 to 35 somites (*Street, 1937*).

### The Nasal Cavity and the Nares

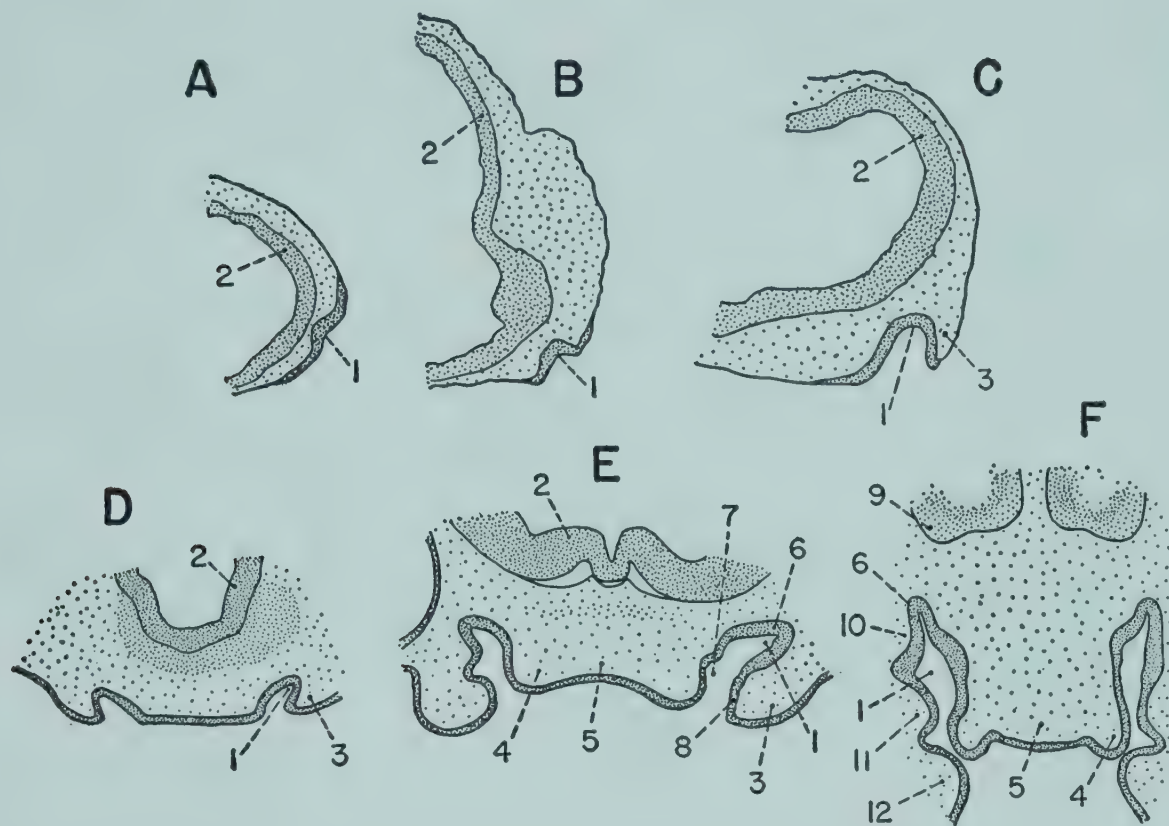
In birds, the bilateral olfactory placodes appear after the formation of the lens placodes. The olfactory organ is thus the last of the three special sense organs to begin development, rather than the second, as in other vertebrates. This change in order is due not to delay in the formation of the olfactory primordia, but to the precociousness of lens development in birds (*Cohn, 1903; Peter, 1906*).

The olfactory placodes form on the lateral walls of the head, anterior to the eyes, and are first seen at the 23- or 24-somite stage in the chick embryo (*Cohn, 1903*) and the duck (*Anas platyrhynchos*) embryo (*Wijhe, 1886*). In the embryo of the zebra parakeet (*Melopsittacus undulatus*), they are said to appear at the 25- or 26-somite stage (*Abraham, 1901*). The placodes consist of high cylindrical cells whose nuclei lie basally. At the edges of the placodes the cells become lower and taper down into the surrounding ectoderm without a sharp transition (*Putelli, 1889; Cohn, 1903*).

Very soon after their initial appearance—during the third incubation day, in the chick (*Kölliker, 1860; Born, 1879*)—the placodes start to sink in and form pits. As they become deeper, they seem to migrate ventralward, no doubt because of the rapid growth of the forebrain above them (Fig. 165-A, B, and C). They have reached the junction of the lateral and ventral walls of the head at the end of the chick's third incubation day (cf. Fig. 165-C), and on the fifth day in the zebra parakeet, *Melopsittacus undulatus* (*Dieulafé, 1905*). In the 4-day chick, they are located on the ventral surface of the head (Fig. 165-D) and are considerably deeper (Fig. 166-A<sub>1</sub> and A<sub>2</sub>). Their open ends are now more medial in position than their fundi (*Preobraschensky, 1892*). A transitory depression found in the 4- and 5-day chick embryo on the medial wall of each pit (Fig. 165-E) is interpreted as a rudimentary Jacobson's organ (*Cohn, 1903*). A distinct anlage of Jacobson's organ in the black-headed gull (*Larus ridibundus*) and in the European coot (*Fulica atra*) persists up to the stage just prior to hatching (*Slabý, 1955*). In the black-headed gull the organ just before hatching is a long and blind canaliculus strewn with stratiform cylindrical or cubical epithelium connected with the lumen of the epithelial nasal tubulus by its rostral end.



The initial invagination of the nasal pits is produced through active mitosis and proliferation of the placodal ectoderm (Cohn, 1903), which eventually gives rise to the sensory or olfactory epithelium and to the olfactory nerves (see Chapter 4). Before all the material of the placodes has invaginated, the mesodermal tissue surrounding the nasal pits starts to increase in mass and to grow out on the lateral and medial sides of each pit (cf. Fig. 165-C, D, and E). The pits thenceforth increase in depth passively.



**Fig. 165.** Early stages in the development of the nose in the chick embryo, shown in semidiagrammatic representations of cross sections. (Redrawn with modifications A, B, and C, after Cohn, 1903; D, E, F, after Preobraschensky, 1892.)

A, B, C, the invagination and apparent migration of one of the nasal pits during the third day of incubation ( $\times 28$ ); D, E, F, sections taken at 4, 5, and 6 days of incubation, respectively ( $\times 16$ ).

1, nasal pit; 2, brain; 3, external nasal process; 4, internal nasal process; 5, frontal process; 6, sensory epithelium of olfactory region; 7, epithelium of respiratory region; 8, rudimentary Jacobson's organ; 9, olfactory bulb of brain; 10, superior concha; 11, inferior concha; 12, vestibular concha.

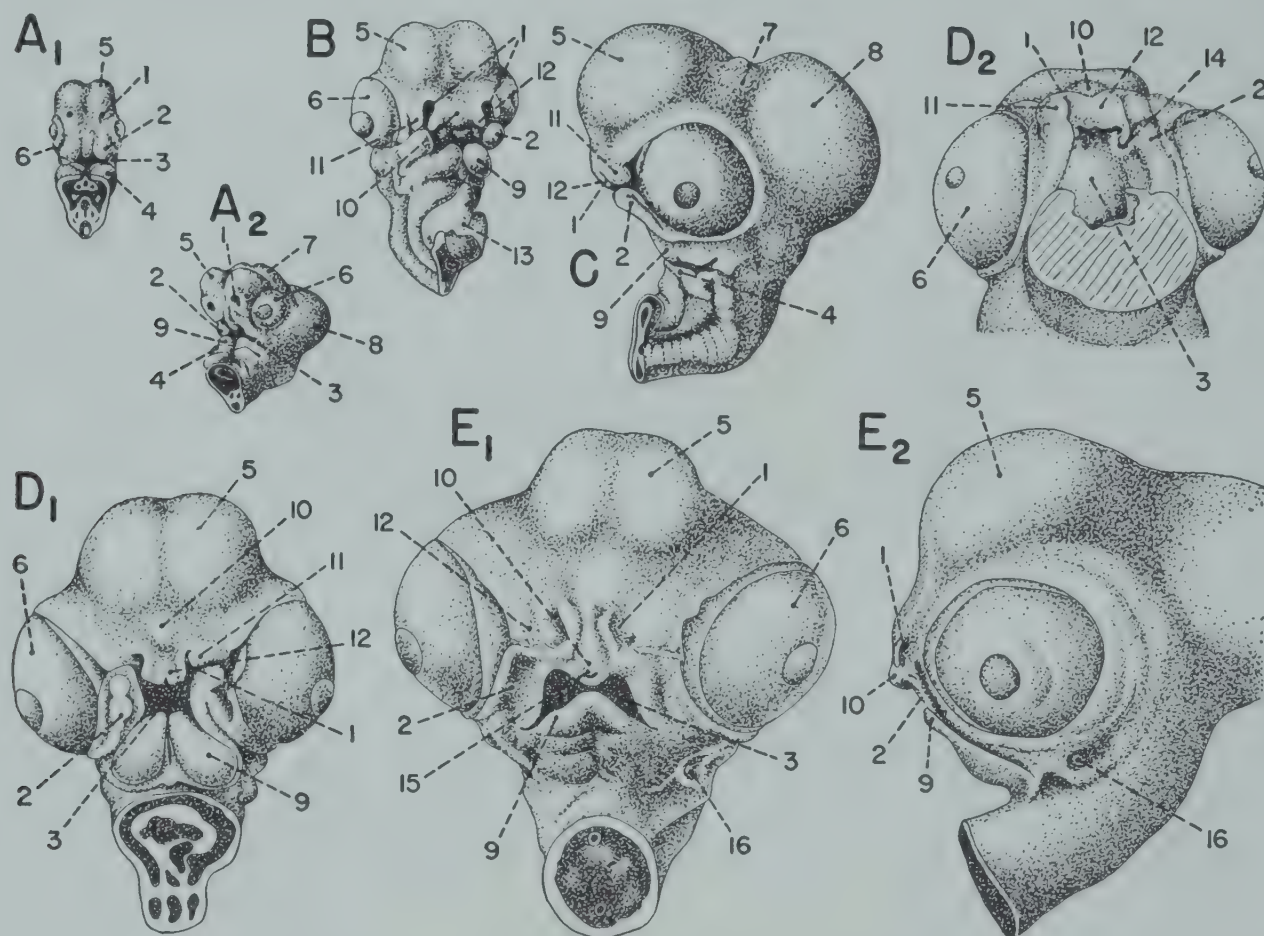
The mesodermal protuberances adjacent to the nasal pits are known as the external and internal nasal processes, in accordance with their respective positions relative to the pits. The internal nasal processes are actually integral parts of the frontal process (Fig. 165-E and F), which is the large medial mass of tissue between the forebrain and the primitive oral cavity.

The outgrowth of the nasal processes around the nasal depressions deepens and narrows the latter into grooves, which are open caudally and are lined in their more external portion with ectoderm of superficial,



extraplacodal origin. In this region, which will give rise to the respiratory passages of the nose, the ectoderm soon differentiates a deeper layer of cuboidal cells and a superficial layer of flattened cells (*Preobraschensky*, 1892).

As the nasal processes continue to enlarge, the external aperture of each nasal pit, as seen from the ventral side, is transformed into a slit open at



**Fig. 166.** The development of the external nares and the primordium of the upper beak in the 4- to 8-day chick embryo, as seen externally. (Redrawn with modifications  $A_1$ ,  $A_2$ ,  $B$ ,  $C$ ,  $D_1$ ,  $E_1$ , and  $E_2$ , after Duval, 1889;  $D_2$ , after Born, 1879.)

$A_1$ , ventral or frontal view of the head of a 96-hour embryo ( $\times 10$ );  $A_2$ , three-quarter view of the same ( $\times 10$ );  $B$ , frontal view of a 5.5-day embryo's head ( $\times 10$ );  $C$ , side view of a 6-day embryo's head ( $\times 8$ );  $D_1$ , frontal view of a 7-day embryo's head ( $\times 6$ );  $D_2$ , frontal view of the head of a 6-day embryo slightly better developed than the 7-day embryo shown in  $D_1$ , with the mandibular processes removed to reveal the interior of the buccal cavity ( $\times 6$ );  $E_1$ , frontal view of an 8-day embryo's head ( $\times 5$ );  $E_2$ , side view of the same ( $\times 5$ ).

1, nasal pit or external nasal opening; 2, maxillary process of first visceral arch; 3, buccal cavity; 4, second visceral arch; 5, telencephalon; 6, eye; 7, diencephalon; 8, mesencephalon; 9, mandibular process of first visceral arch; 10, frontal process; 11, external nasal process; 12, internal nasal process; 13, heart; 14, choana; 15, egg tooth; 16, external auditory meatus.

the end nearest the oral cavity but surrounded elsewhere by a bulging arch of tissue (Fig. 166-B). Meantime (on the third and fourth incubation days, in the chick), the maxillary process has started to increase in size. It progressively narrows the space between itself and the external nasal process (Fig. 166-C) until it contacts the free or caudal edge of the latter



and fuses with it (Fig. 166-D<sub>1</sub>). The free or caudal border of the internal nasal process then comes into contact with the combined maxillary and outer nasal processes and fuses with them at the end of the fifth day or the beginning of the sixth (Kölliker, 1860; Born, 1879; Peter, 1906). The nasal pits, or grooves, thus become canals leading from the outside into the forming buccal cavity, into which they now open by means of the primitive choanae (Fig. 166-D<sub>2</sub>). The external apertures, for the first time, are surrounded by tissue on all sides.

The palatine processes of the maxillary processes start to grow medially on the chick's fifth day; they hide the choanae from view on the eighth day. At this time, the palatine processes are two internal ridges along the entire length of the upper beak. Two thin plates now begin to extend

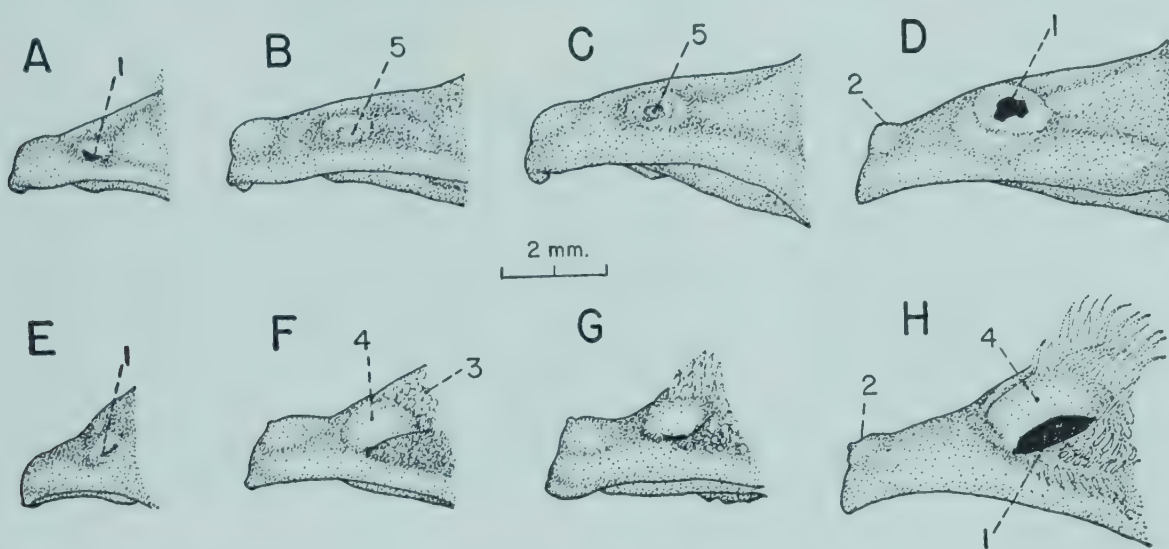


Fig. 167. Late stages in the development of the upper beak and the external nares in the European quail, *Coturnix coturnix*, and the starling, *Sturnus vulgaris*. (Redrawn with modifications after Weber, 1950.)

A, B, C, D, left lateral views of the beak of the starling embryo of 10, 13, 16, and 18 days' incubation, respectively; E, F, G, H, left lateral views of the beak of the quail embryo of 7, 9, 11, and 13 days' incubation, respectively. All in scale.

1, external naris; 2, egg tooth; 3, feather primordia; 4, operculum; 5, obstructed naris.

medialward from the palatine processes. On the eleventh day, these plates meet in the midline without fusing and thus form the split palate characteristic of birds (Born, 1879).

The fused maxillary, nasal, and frontal processes grow out to form the upper beak (Fig. 166-E<sub>1</sub> and E<sub>2</sub>; Fig. 167), although the lateral portions of both the maxillary and the external nasal process contribute tissue to the eyelid (Born, 1879). With the growth of the beak, the nasal fossae are elongated, especially in the vestibular region. Meantime, they continue to approach each other medially as the mesenchymatous tissue separating them becomes narrower in the course of forming the nasal septum (see Chapter 12).

The forward growth of the upper beak between the external nares shifts them to a more lateral position, and their shape and direction are



altered somewhat (cf. Fig 167). The nares are elongated in the 6-day chick and become shorter and rounder on the eighth and ninth days of incubation (Born, 1879).

Very shortly after the nasal apertures are surrounded by tissue, they become closed completely and remain so until hatching time (Born, 1879; Preobraschensky, 1892; Abraham, 1901; Dieulafé, 1905; Peter, 1906; Branca, 1908; Street, 1937; Weber, 1950). The epithelial portions of the inner and outer nasal processes grow together (Fig. 168-A; Fig. 169-A) and close

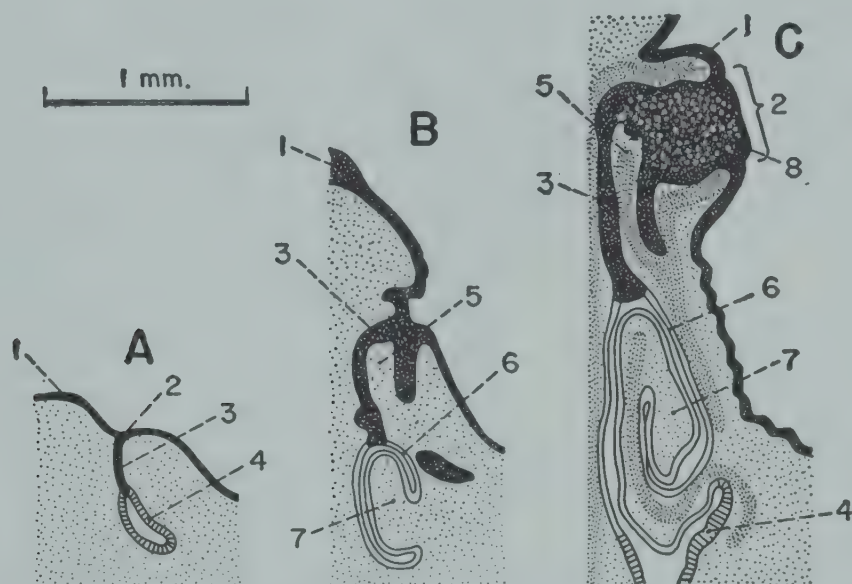


Fig. 168. Stages in the development of the nasal cavity in the starling (*Sturnus vulgaris*) embryo, as seen in horizontal sections taken through the right half of the upper beak. (Redrawn from Weber, 1950.)

A, at 5 days; B, at 7 days; C, at 10 days of incubation. All in scale.

1, epidermis; 2, external nasal aperture; 3, epithelial tissue in vestibule of nasal cavity; 4, sensory epithelium; 5, vestibular concha; 6, respiratory epithelium; 7, inferior concha; 8, region of granulation of epithelial tissue.

the vestibule. The obstruction of the vestibule is clearly visible from the outside in some birds (cf. Fig. 167-A to D), such as the starling (*Sturnus vulgaris*), but is not easily seen in others (cf. Fig. 167-E to H), including the chick and the European quail (*Coturnix coturnix*). The proliferation of the basally located epithelial cells produces a mass of tissue that fills the future vestibular portion of the cavity (Fig. 168-B; Fig. 169-B) and increases in size until approximately the midpoint of incubation. At this time, in the region nearest the external nares the cells midway between the basal layers begin to exhibit granulations (Fig. 168-C; Fig. 169-C), which gradually grow larger; and the nuclei of these cells show early signs of degeneration (Branca, 1908; Weber, 1950). The zone of granulation extends itself inward and throughout the entire epithelial mass (Fig. 169-D and E), leaving only the basal layer and a few intermediate layers unaffected. The granular cells rapidly become vacuolar and hyaline a short time before hatching. This change has been observed to occur only 1 or 2 days prior to the hatching date in the starling (*Sturnus vulgaris*), the



European quail (*Coturnix coturnix*), the zebra parakeet (*Melopsittacus undulatus*), and the alpine swift (*Apus melba*), whose incubation periods range from 13 to 20 days (Weber, 1950). On either the day of hatching or the preceding day, the entire tissue plug shrinks away from the walls of the vestibule and degenerates (Fig. 169-F). The line of separation lies a few cell layers above the basal cells.

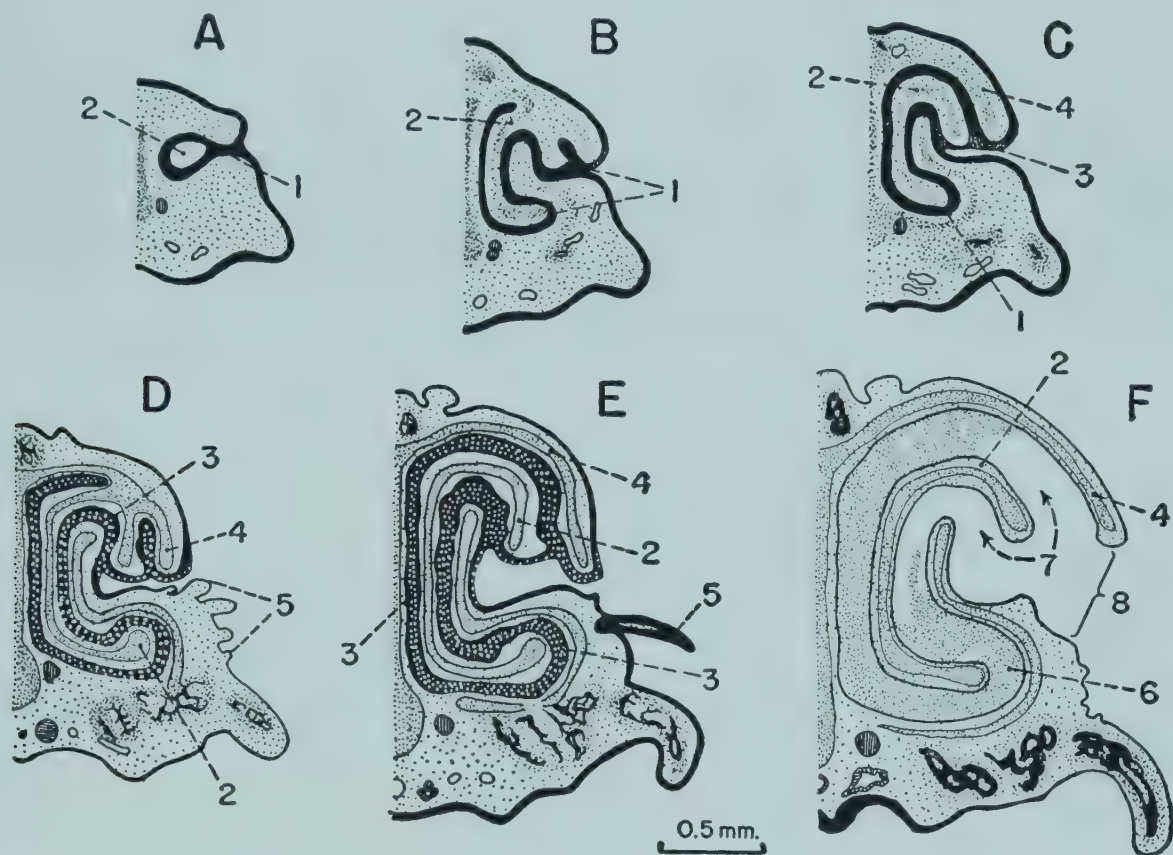


Fig. 169. Successive stages in the development and degeneration of the tissue obstructing the nasal vestibule in the embryo of the European quail (*Coturnix coturnix*), as seen in cross sections through the upper beak. (Redrawn with modifications after Weber, 1950.)

A, at 9 days; B, at 10 days; C, at 12 days; D, at 14 days; E, at 16 days; F, at 18 days' incubation. All in scale.

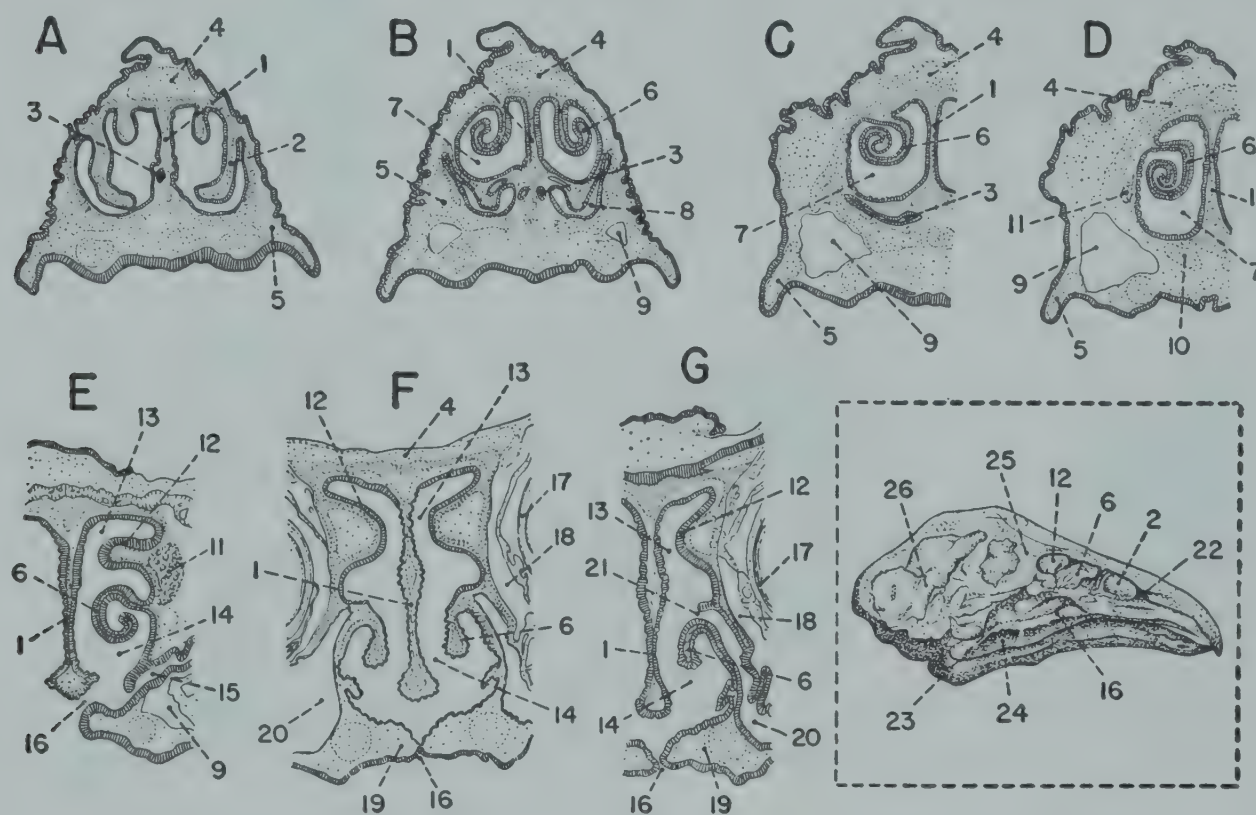
1, epithelial tissue obstructing vestibule; 2, vestibular concha; 3, granular epithelial tissue; 4, operculum; 5, feather anlagen; 6, remnant of obstructing tissue; 7, vestibule; 8, external naris.

### The Conchae

The inferior (primary or middle) concha is the first to appear. Its primordium is a rounded protuberance, found in the 4-day chick projecting into the nasal cavity from the lateral wall (Peter, 1906; Street, 1937). A day or so later, the development of the superior (secondary) concha is initiated in the same manner in the fundus of the nasal cavity. Cohn (1903), however, signified that this concha is the first to start development. The vestibular concha of the chick (cf. Fig. 165-F) begins to form at 6 or 7 days of incubation (Born, 1879; Cohn, 1903; Peter, 1906). Its development in the quail (*Coturnix coturnix*) may be followed in Fig. 169.



The middle concha has the form of a pediculated lamina in the 8-day zebra parakeet, *Melopsittacus undulatus*, embryo (Dieulafé, 1905) and in the duck, *Anas platyrhynchos* (Dieulafé, 1905), and European quail, *Coturnix coturnix* (Weber, 1950), embryos of 10 days' incubation. According to the account given by Dieulafé (1905), this concha is formed as the result of "fissuration"; that is, narrow diverticuli of the lumen of the



**Fig. 170.** The nasal cavity of the fully developed chick embryo, as seen in cross sections of the upper beak. The sections A to G are taken at successively more posterior levels. (Redrawn after Mihalkovics, 1898.)

A, section through the vestibule; B to G, sections through the olfactory region and the superior concha. All  $\times 7$ .

*Insert:* a view of the left lateral wall of the nasal cavity of the adult chicken, as seen from the medial side after removal of the nasal septum and the entire right half of the beak ( $\times 2$ ).

1, nasal septum; 2, vestibular concha; 3, duct of lateral nasal gland; 4, nasal bone; 5, maxillary bone; 6, inferior concha; 7, nasal cavity; 8, inferior recess of nasal cavity; 9, palatine sinus; 10, intermaxillary bone; 11, lateral nasal gland; 12, superior concha; 13, olfactory region of nasal cavity; 14, respiratory region of nasal cavity; 15, lateral palatine fissure; 16, median palatine fissure; 17, eye; 18, orbital sinus; 19, palate; 20, maxillary sinus; 21, aperture of maxillary sinus; 22, naris; 23, choana; 24, nasopharyngeal meatus; 25, lamina cribrosa; 26, brain.

nasal cavity penetrate into the walls around the tissue that is to become the concha and shape it as it grows out.

The elongation of the nasal cavity causes the spatial relationships between the conchae to be somewhat altered. The definitive appearance of the conchae in the chicken is shown in Fig. 170 both in cross section and in a longitudinal section of the upper beak.



### The Nasal Epithelium

It has been stated that the sensory or olfactory epithelium is confined from the beginning to the superior concha and the olfactory region of the nasal cavity (*Preobraschensky*, 1892). It has also been indicated, however, that sensory epithelium is originally present over a portion of the inferior concha but soon recedes from it (*Cohn*, 1903; *Street*, 1937; *Weber*, 1950). In the 10-day chick embryo, the olfactory epithelium is a pseudostratified, generalized sensory type made up of supporting cells and olfactory cells side by side, with an innermost region composed of nerve fibers and the processes of supporting cells; a region of low sensory epithelium forms a zone of transition between the olfactory epithelium and the stratified epithelium of the inferior concha. Later, the small nuclei of the supporting cells form a band outside the large olfactory nuclei, and olfactory hairs and mucus-secreting Bowman's glands develop (*Street*, 1937). From the beginning, the epithelium of the vestibule and the vestibular concha is nonsensory (*Street*, 1937); it arises from the nasal epithelial layer and the cells between the latter and the obstructing plug of tissue. Even before the separation and degeneration of the tissue plug, the intermediate cells begin to flatten. Very shortly before hatching, they become keratinized (*Branca*, 1908; *Street*, 1937; *Weber*, 1950).

### The Nasal Glands

The lateral nasal gland starts to develop in the chick on the eighth incubation day as a solid bud of tissue in the septal wall of the vestibule. It grows, in the form of a solid cord of cells, beneath the floor of the vestibule and attains the lateral wall of the nasal cavity on the tenth day. It then elaborates gland tubules at the level of the inferior concha. The solid cord of cells develops a lumen and is recognizable as the duct of the gland by the fourteenth day (*Born*, 1879). According to *Ganin* (1890), the nasal gland of the chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), grouse (*Bonasa umbellus*), and partridge (*Perdix perdix*) possesses no duct in the lateral wall of the nasal cavity (cf. Fig. 170). In many other birds (such as *Anas*, *Anser*, *Columba*, *Picus*, *Passer*, *Carduelis*, *Emberiza*, *Otocoris*, *Parus*, *Regulus*, *Limosa*, *Corvus*, *Pica*, *Frugilegus*), the lateral duct lies in the outer wall of the nasal cavity and has its opening at the level of the posterior border of the vestibular concha. In the pigeon (*Columba livia*), this duct first appears in the middle of the eighth incubation day as a solid bud of tissue in the outer epithelial wall of the vestibule. It grows posteriorly close to the inner surface of the nasal cavity. The medial duct of the gland arises a day later and develops in the same manner as in the chicken.







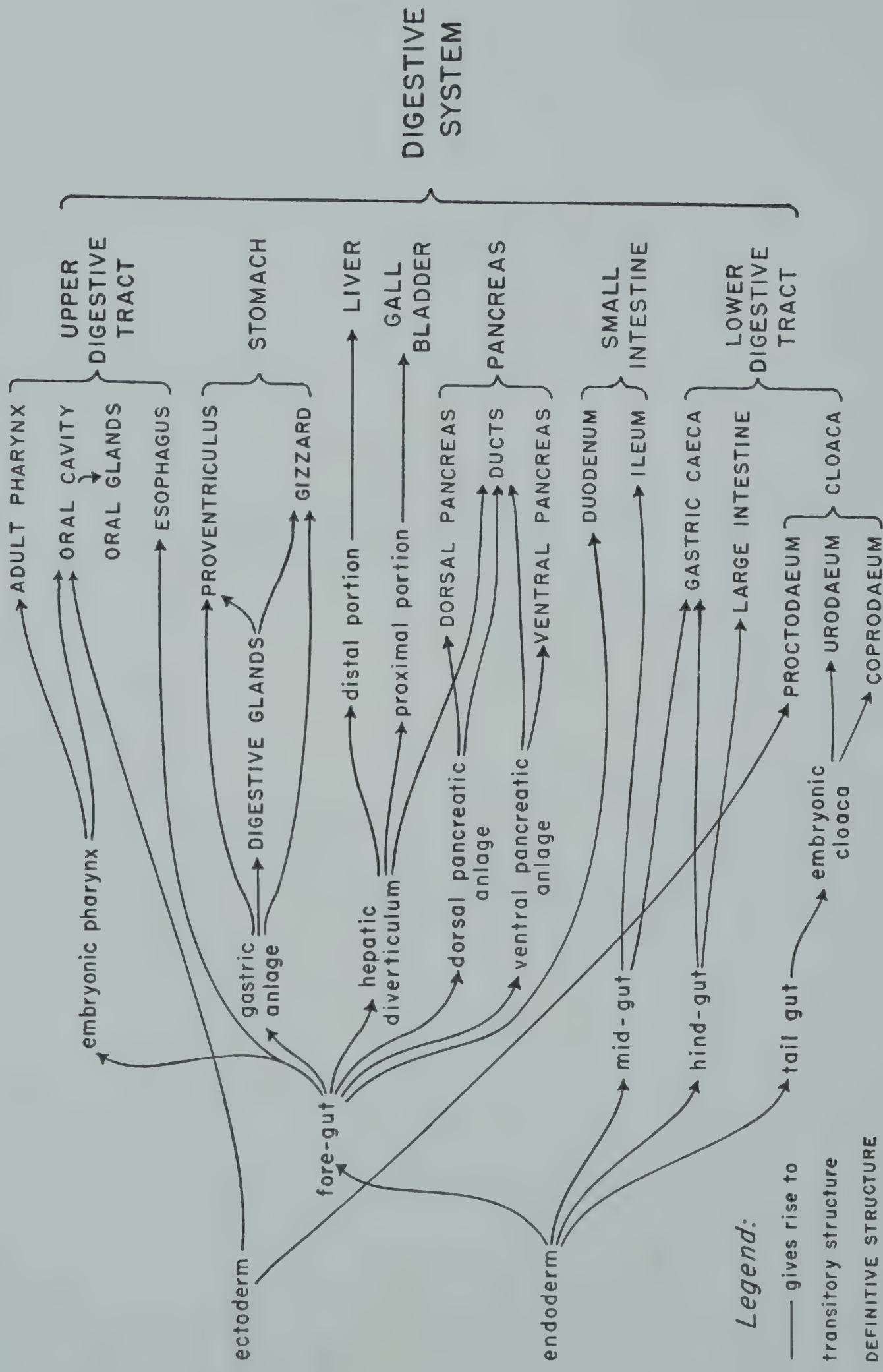
## CHAPTER SIX

# *The Digestive System*

*It is a system of digestion*

*Which has strong motives, vital drive  
To fill the want without a question,  
To keep the organism alive.*







# THE DIGESTIVE SYSTEM

The digestive tract in birds exhibits certain distinctive features. A localized dilatation, known as the crop, occurs along the course of the esophagus. The stomach is subdivided into two portions, the proventriculus or glandular stomach, which supplies the digestive juices, and the gizzard or muscular stomach, which, in the absence of teeth, performs the function of grinding ingested food. The alimentary canal terminates in a chamber, the cloaca, into which open the genital and urinary ducts. Throughout the avian order, there is much variability in the structure of the digestive tract; for example, the caecum may be large, small, double, or absent, and may assume a great variety of forms.

The portion of the digestive system that functions in the actual process of digesting and assimilating food is derived from endoderm. The embryonic structure that gives rise to this part of the alimentary canal and to the accessory digestive glands (liver and pancreas) is a simple tube consisting of endoderm surrounded by splanchnic mesoderm and formed by the fusion in the midline of two folds of splanchnopleure coming from either side. The muscular and connective tissue tunics of the digestive tract originate from the investing layer of mesoderm. A number of organs that do not participate in digestion also take their origin from the primitive gut tube. Small portions of the oral cavity and cloaca are derived from ectoderm.

The embryonic gut is more or less arbitrarily divided into three portions. The most anterior, and the first to appear, is the fore-gut, formed from anterior to posterior and delimited caudally by the primordia of the liver. The second portion to be established is the posterior segment, or hind-gut, formed from posterior to anterior and delimited cranially by the caecal primordia. The segment intervening between the fore-gut and the hind-gut is known as the mid-gut.

The fore-gut has the largest number of derivatives. Some of the structures originating from it are integral parts of the digestive system, such as the esophagus, the crop, the proventriculus, the gizzard, and most of the oral cavity and duodenum. Other derivatives of the fore-gut remain connected to the alimentary tract by ducts and have both digestive and other functions: the liver, gall bladder, and pancreas comprise this group. A third group of fore-gut derivatives breaks away partially or completely from the digestive tract and retains no physiological significance in alimentation.



This group includes the respiratory system (larynx, trachea, syrinx, bronchi, lungs, and air sacs) which is connected with the alimentary canal only at the glottis; the development of this system is described in Chapter 7. Also in the third group are the tympanic cavities and the Eustachian tubes, which open into the pharyngeal region (see Chapter 5); and the thyroid, parathyroid, and thymus glands and the ultimobranchial bodies, which do not communicate with the alimentary canal and are discussed in Chapter 11.

The mid-gut gives rise to the small intestine, except for the duodenum. During embryonic life, the mid-gut is continuous with the yolk sac at the umbilicus. The cloaca, rectum, large intestine, and caecal appendages originate from the hind-gut.

### GUT POTENCIES DURING EARLY STAGES

The primary endoderm that delaminates from the epiblast (see Chapter 3) does not seem to possess the capacity to produce gut. Digestive tract tissues do not differentiate in chorioallantoic grafts of endoderm (with or without adherent mesoderm) taken from the chick blastoderm before late primitive streak stages (*Waddington, 1932; Hunt, 1934, 1937a*). On the other hand, grafted mesectoderm (ectoderm plus adherent mesoderm), including the mesectoderm of the streak itself, gives rise to gut and its derivatives until the definitive primitive streak or the head-process stage, when grafted mesendoderm begins to show the ability to differentiate as digestive tract (*Hunt, 1934, 1937a; Rudnick, 1935, 1952*). Assuming that primary endoderm is not regenerated from ectoderm in grafts, these findings support the conclusion that definitive endoderm, endowed with gut-forming potency, is normally derived from cells invaginated through the primitive streak. In fact, vitally stained ectodermal cells have been seen to enter the primitive streak and occasionally pass into the endodermal rather than the mesodermal layer (*Hunt, 1937b*). The definitive endoderm probably is not established until, or shortly before, the head-process stage (*Hunt, 1937a*). Even as late as head-process and early somite stages, however, the endoderm does not seem to act as a developmental unit or to have a high degree of autonomy. Its differentiation into gut in grafts appears to depend largely upon the presence of mesoderm (*Rudnick and Rawles, 1937; Rudnick, 1944*).

The distribution of potencies for gut and its derivatives changes during the early developmental period. In the unincubated blastoderm, liver and gut potencies are found chiefly in the posterior half of the blastoderm, and potencies for such fore-gut derivatives as esophagus, proventriculus, thyroid, trachea, and pharynx are more or less concentrated in the median posterior quadrant (*Butler, 1935*), where the primitive streak will appear. Subsequently, in primitive streak and head-process stages, the capacity to



form gut is present at all primitive streak levels (*Hunt, 1929, 1931a, 1932*), but potencies for fore-gut derivatives become localized anterior to the midlevel of the streak (*Dalton, 1935; Rawles, 1936; Rudnick and Rawles, 1937*). At the stage of the definitive streak, potencies for proventriculus, respiratory tissue, and liver are demonstrable at a level extending about 0.3 mm. to 0.4 mm. anterior and posterior to the node, and future thyroid-forming cells are found only anterior to the node (*Hunt, 1932*). In head-process blastoderms, before the formation of the head fold, the fields for fore-gut derivatives are coextensive anteriorly with the head-process, while still extending about 0.3 mm. behind the node (*Rawles, 1936*). At this time, thyroid and liver potencies have become localized in two bilateral areas and are no longer present medially (*Rudnick, 1932; Willier and Rawles, 1935; Rawles, 1936*).

The actual incorporation of carbon-marked endodermal cells into the fore-gut has been followed in blastoderms explanted (endoderm side up) to plasma clots. It was found that all cells destined for the fore-gut originate in an oval area approximately coextensive anteriorly with the prechordal mesoderm. This oval area surrounds the node at the definitive primitive streak stage but lies around the head-process after the node has started to regress (*Bellairs, 1953a*). There is thus an approximate correspondence between the presumptive fore-gut area and the region where potencies for fore-gut derivatives have been demonstrated. Marked endodermal cells lateral to the fore-gut area enter the yolk sac endoderm, and those posterior to it migrate posteriorly and posterolaterally (*Bellairs, 1953a*).

Potency for small and large intestine is found behind the fore-gut area in primitive streak, head-process, and early somite stages (*Dalton, 1935; Rawles, 1936; Hunt, 1937a; Rudnick and Rawles, 1937*); the potencies for rectum and cloaca are localized most posteriorly of all (*Dalton, 1935*). It is thus apparent that there is an anteroposterior arrangement of potential areas corresponding to the definitive order of the various parts of the digestive tract.

## THE FORMATION AND CLOSURE OF THE PRIMITIVE GUT TUBE

The establishment of a tubular gut from the originally flat endoderm of the area pellucida occurs in association with the elevation and constriction of the embryo from the blastoderm. Detachment of the body and closure of the gut tube begin simultaneously at the anterior end of the embryonic area and then proceed caudad from the head. Somewhat later, the same two processes begin at the caudal end of the body and progress cephalad.

The fore-gut, being the most cephalic part of the primitive gut tube, is the first portion to form. Development of the fore-gut begins when all three



germ layers become elevated along the anterior and anterolateral borders of the prechordal mesoderm (Adelmann, 1922). A ridge is thus formed in the shape of a crescent open posteriorly. This ridge, which is the primordium of the head, is bounded externally by the head fold, an indentation in the surface of the blastoderm. The initial differentiation of the cephalic region is probably due chiefly to the rapid anterior growth of the neural plate (Gruenwald, 1941b), but it is not likely that the endoderm

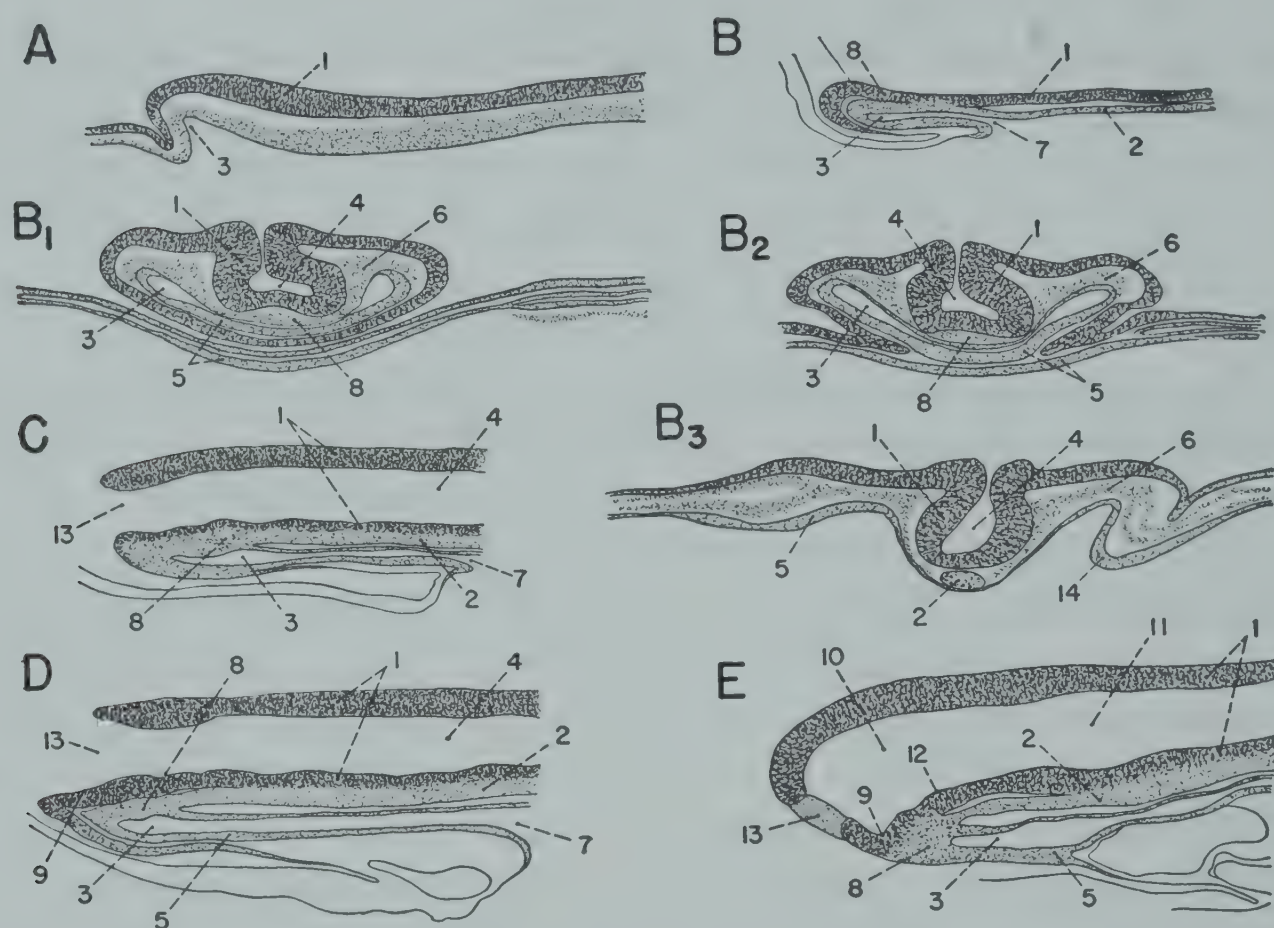


Fig. 171. Early stages in the formation and closure of the fore-gut in the chick embryo. (Redrawn with modifications A, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, after Duval, 1889; B, C, D, and E, after Adelmann, 1922.)

A, sagittal section, 1- to 2-somite stage; B, the same, 4-somite stage; B<sub>1</sub>, cross section through closed portion of fore-gut, 4-somite stage; B<sub>2</sub>, the same, more posteriorly; B<sub>3</sub>, the same, posterior to the anterior intestinal portal, where folds are forming endodermal trough; C, sagittal section, 6- to 7-somite stage; D, the same, 8- to 9-somite stage; E, the same, 13- to 14-somite stage. All  $\times 40$ .

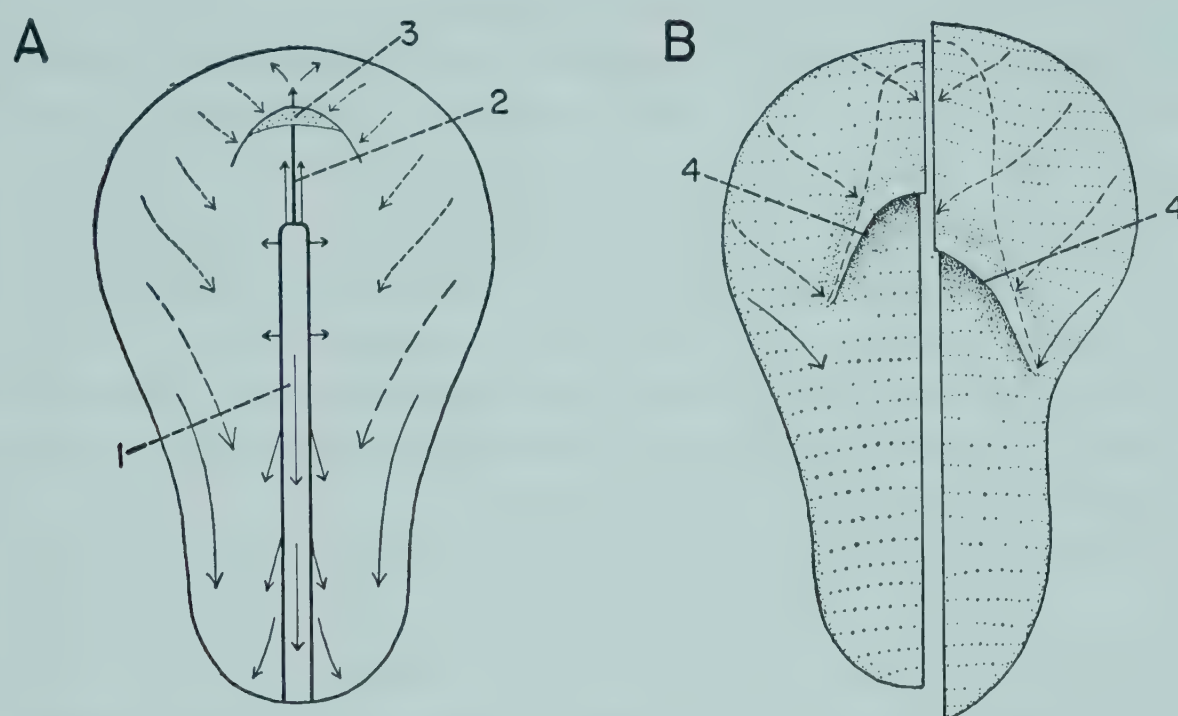
1, neural tube; 2, notochord; 3, pharynx; 4, neural groove; 5, endoderm; 6, mesoderm; 7, anterior intestinal portal; 8, prechordal plate; 9, infundibulum; 10, forebrain; 11, mid-brain; 12, tuberculum posterius; 13, neuropore; 14, border of intestine.

is carried passively into the head. The movement of carbon marks placed on the endoderm has demonstrated an active forward migration of the latter tissue in the midline, anterior to the primitive streak (cf. Fig. 172-A), during head-process and early head fold stages (Bellairs, 1953b). Furthermore, the observation of spontaneous and experimentally produced abnormalities (Waddington and Cohen, 1936; Gruenwald, 1941b) indicates that fore-gut formation and head formation are independent of each other.

At the stage of 1 or 2 somites, the head has begun to project forward,



and the head fold beneath it has deepened (Fig. 171-A). At this time, the fore-gut is merely the endodermal lining of the head, and, as seen in embryos of the chicken, the zebra parakeet (*Melopsittacus undulatus*), and the European lapwing (*Vanellus vanellus*), it has the form of a broad, shallow pocket (cf. Fig. 172-A), closed anteriorly and open to the yolk posteriorly (Goette, 1867; His, 1868; Braun, 1882; Duval, 1889; Keibel and Abraham, 1900; Abraham, 1901; Grosser and Tandler, 1909). The roof



**Fig. 172.** A diagrammatic representation of the developing avian fore-gut, with the broken arrows showing three-dimensional movements, and the solid arrows showing two-dimensional movements. (Redrawn with modifications after Bellairs, 1953b.)

A, shows morphogenetic movement in the endoderm of the area pellucida at the beginning of fore-gut formation; B, two stages in the closure of the fore-gut.

1, primitive streak; 2, head-process; 3, fore-gut; 4, anterior intestinal portal.

of the fore-gut adheres closely to the prechordal mesoderm, but its floor is contiguous to the external ectoderm of the head. The floor and the anterior wall of the fore-gut are thick and composed of tall columnar cells (cf. Fig. 171-B), but the roof abruptly becomes thin behind the anterior thickened zone (Adelmann, 1922). The roof is concave dorsally (cf. Fig. 171-B<sub>1</sub> and B<sub>2</sub>) and is continuous posteriorly with the thin endoderm that lies below the notochord and the paraxial mesoderm. The thick floor of the fore-gut is continuous for a short distance posteriorly with a zone of similarly thickened endoderm (cf. Fig. 171-B<sub>3</sub>) underlying the anterior portion of the splanchnic mesoderm on either side (Duval, 1889; Bellairs, 1953a).

The fore-gut is elongated backward by a process that begins when the bilateral thickened portions of the endoderm, closely followed by the splanchnic mesoderm, start to swing ventromedially. An inverted endodermal trough is thus produced (cf. Fig. 171-B<sub>3</sub>). As soon as the sides of the trough complete their ventromedial movement and come into contact



with each other in the midline, they fuse, lose their connection with the extraembryonic endoderm, and form a continuation of the floor of the fore-gut. The floor, like the roof, is concave dorsally. The roof, originally adherent to the chorda, gradually separates from the latter, beginning posteriorly (*Adelmann, 1922*), as shown in Fig. 171-C, D, and E. The posterior edge of the closed fore-gut is known as the anterior intestinal portal and has the shape of a crescent with convexity directed anteriorly (cf. Fig. 172).

Changes in the position of carbon marks on the endoderm indicate that the initial formation and the elongation of the fore-gut floor may be due to an obliquely medial and posterior movement of endodermal material on either side of the presumptive fore-gut roof (Fig. 172-A and B). Such bilateral movements would tend to produce folds perpendicular to their direction. As development proceeds, the oblique movements occur at increasingly posterior levels, and the ventral closure of the fore-gut is thus continued caudad. In the posterior part of the area pellucida, however, the oblique movements disappear and are succeeded by a displacement of cells directly to the rear (*Bellairs, 1953b*).

The closure of the fore-gut and the detachment of the head from the blastoderm take place at approximately the same rate during the period when the first four or five somites are forming (*Gruenwald, 1941b*). Within this time, the chick's fore-gut attains a length of about 350 microns. By the 6-somite stage (Fig. 171-C), it has increased to a length of 450 microns (*Keibel and Abraham, 1900*). The closure of the fore-gut now begins to outstrip the backward extension of the ectodermal portion of the head fold (*Goette, 1867; Duval, 1889*). As the walls of the inverted endodermal trough move ventromedially, the closely attached splanchnic mesoderm, enclosing an extension of the coelomic cavity, is also brought ventromedially. As soon as the distance begins to increase between the ectodermal and endodermal components of the head fold, the mesoderm from either side occupies the intervening space. Consequently, at the 7- or 8-somite stage (Fig. 171-D), the pericardial cavity and the fusing heart tubes are found ventral to the fore-gut and the anterior intestinal portal is separated from the ectodermal head fold (*Duval, 1889; Adelmann, 1922*). The latter no longer parallels the anterior intestinal portal but forms a subcephalic ectodermal pocket, which, when viewed from above, resembles a crescent with convexity directed posteriorly. The most cranial part of the fore-gut remains intimately attached to the ectoderm to form the oral plate or pharyngeal membrane (Fig. 171-E).

From now on, the anterior intestinal portal lies directly behind the level where the vitelline veins enter the body and fuse to form the ductus venosus, or omphalomesenteric trunk. The position of the anterior intestinal portal relative to these veins remains the same merely because the



veins are also migrating posteriorly as the result of the closure of the fore-gut.

Between the 7-somite and the 27-somite stage, the length of the chick's fore-gut increases from 560 microns to 900 or 1000 microns (*Keibel and Abraham, 1900*), and the open, inverted trough of thickened endoderm extends itself caudad from the anterior intestinal portal as far as the level of the vitelline arteries. Elongation of the fore-gut proceeds at a similar rate in the European lapwing (*Vanellus vanellus*) embryo, which is comparable in size to the chick embryo (*Grosser and Tandler, 1909*). In the zebra parakeet (*Melopsittacus undulatus*), a smaller bird, the fore-gut is almost as long as the chick's, at least from the 7- to the 15-somite stage (*Abraham, 1901*).

The formation of the fore-gut is considered to be complete between the stages of 20 and 27 somites, reached in the chick embryo at the end of the second day or early in the third day. The liver primordium, which conventionally marks the transition from fore-gut to mid-gut (*Goette, 1867*), appears during these stages as a ventrad expansion of the gut lumen immediately cranial to the anterior intestinal portal and directly behind the sinus venosus (*His, 1868; Duval, 1889; Brouha, 1898b; Keibel and Abraham, 1900; Abraham, 1901; Grosser and Tandler, 1909*).

The first indication of gut formation at the caudal end of the body is seen at approximately the same time that closure of the fore-gut is completed. As late as the 20-somite stage, the endoderm cannot be distinguished directly beneath the undifferentiated mass of the incipient tail bud (*Holmdahl, 1939b*). Immediately behind the tail bud can be seen the remnant of the posterior part of the primitive streak (*Gasser, 1877c, 1880; Gaertner, 1949*), in which the mesodermal layer is unrecognizable (*Gasser, 1874, 1880*). The tail bud now begins to swell, both dorsally and ventrally. As it does so, a medially directed fold forms in the splanchnopleure on either side of the vestigial primitive streak and the caudal part of the tail bud. In embryos of 20 to 27 somites (*Duval, 1889; Zwillling, 1946*), the endoderm of these two folds has fused in the midline and has thus produced a wide, flat pocket beneath the primitive streak. This pocket, whose blind end is directed dorsocaudad at the start, is the primordium of the allantois (see Chapter 13). Although differentiated endoderm lines the entire allantoic diverticulum, none has as yet appeared on the ventral surface of the tail bud (*Holmdahl, 1939b*). The site of the primitive streak remnant is now rather indefinite but is usually identified with the fundus of the allantoic diverticulum, or with the portion of its cranial (or dorso-cranial) wall adjoining the fundus. In the approximate region of the vestigial primitive streak, the endoderm of the allantoic diverticulum is fused with the surface ectoderm to form the cloacal membrane, or anal plate (*Cadiat, 1878*).



Almost immediately after the appearance of the allantoic pocket, the somatic tail fold becomes recognizable as an indentation in the upper surface of the blastoderm directly behind the tail bud and slightly anterior to the cloacal membrane. Cranial to the tail bud, a second, very shallow endodermal diverticulum soon appears, as if produced passively by the continued ventral swelling of the tail bud mass. This diverticulum is the primordium of the caudal intestine, or tail gut (*Gasser, 1874; Duval, 1889; Gruenwald, 1941b; Zwilling, 1946*), which is a transitory structure.

The dorsal portion of the tail bud now starts to grow rapidly backward and downward, rotating around the ventral portion. The tail, as yet containing no gut cavity (*His, 1868, p. 156*), thus begins to be detached from the blastoderm. Between the 23- and 28-somite stages, the rotational movement of the tail bud changes the positions of the tail gut and allantoic diverticulum so that the former becomes dorsal to the latter and both open anteriorly instead of ventrally (*His, 1868, p. 159*). In the 28- to the 29-somite embryo, endoderm has differentiated over the cranial (formerly ventral) surface of the tail bud, and the tail gut begins to extend caudad into the tail, where its blind extremity merges with the undifferentiated tissue of the tail bud (*Holmdahl, 1939b*). As the tail grows, the tail gut elongates within it. The caudal intestine thus forms by a process distinctly different from the closure of the fore-gut (*Gruenwald, 1941b*).

By the 29- to 32-somite stage (*Duval, 1889; Holmdahl, 1939b; Gruenwald, 1941b*), or early in the chick's third incubation day (*Gasser, 1874*), the continued rotation of the tail has brought the allantois anterior to the tail gut (see Fig. 415 in Chapter 13). The original ventrocaudal wall of the allantois has been stretched out and transformed into a ventral diverticulum of a high endoderm-lined cavity which is continuous posteriorly with the tail gut. This cavity is the embryonic cloaca, the first portion of the hind-gut to form. The cloacal membrane now lies at the caudal boundary of the allantois. The fate of the original dorsocranial wall of the allantois is uncertain (*Zwilling, 1946*).

The hind-gut soon begins to extend itself cranial by the apposition and fusion of two endodermal folds in the midline anterior to the cloaca. The hind-gut opens to the yolk through the posterior intestinal portal, which rapidly becomes joined to the anterior intestinal portal by an uninterrupted endodermal trough. The hind-gut continues to grow by elongation at its anterior end, while the fore-gut, as before, prolongs itself caudad. As the anterior and posterior intestinal portals approach each other, the intervening segment of open intestinal trough is shortened, of course, at each end.

By the middle or end of the chick's third incubation day, the tail gut begins to degenerate. At this time or the beginning of the fourth day, the boundary between the hind-gut and the mid-gut is indicated by the ap-



pearance of the caecal primordia (*Keibel and Abraham, 1900; Maumus, 1902; Kersten, 1912*).

During the first half of the chick's fourth day, the open portion of the gut (the intestinal umbilicus) decreases in length from about 3000 microns to approximately 750 microns (*Kersten, 1912*). By the end of the same day, the tail gut has disappeared (*Keibel and Abraham, 1900*). The entire gut is now closed except for a section of mid-gut about 240 microns long (*Keibel and Abraham, 1900*) extending from the fork of the vitelline veins to a level somewhat forward of the anterior border of the leg buds. This open portion, which is narrow and troughlike, is closed and added to the mid-gut during the course of the next day (*Duval, 1889*). In the middle of the fifth day, the umbilicus is only 150 microns long (*Kersten, 1912*). At this time, the connection between the intestine and the yolk sac is funnel-like, but it is rapidly transformed into the round, tubular yolk stalk (see Chapter 13).

Long before the final closure of the primitive gut, the individual sections of the digestive tract have begun their differentiation.

### THE EMBRYONIC PHARYNX

When the appearance of the first primordium of the liver indicates that the fore-gut is present and closed in its entirety, most of the fore-gut is still within the head and is therefore regarded as the pharynx. The embryonic pharynx, however, is transitory and is quite different from the rather ill-defined pharynx of the adult. Except for its most posterior portion, the pharyngeal part of the embryonic fore-gut is broad and rather flat. It characteristically develops a series of bilaterally paired evaginations, known as pharyngeal or visceral pouches, which are situated between mesodermal thickenings (pharyngeal or visceral arches) and extend laterally toward depressions in the external ectoderm (pharyngeal or visceral furrows). Together, these structures form a complex which obviously corresponds to the branchial or gill region of lower forms. Below the branchial level, the pharynx becomes irregularly oval or round in cross section and tapers into its much narrower esophageal segment. From the embryonic pharynx are derived the adult pharynx; various glands of the cervical region; part of the oral cavity, ear, and esophagus; and the respiratory system, which takes its origin from the ventral wall of the postbranchial portion of the embryonic pharynx.

Six pairs of visceral pouches have been recognized in embryos of a number of avian species, but only the first four are well marked; the others are small and rudimentary. The fifth visceral pouch of the chick was described by Meuron (1886*b*), Bemmelen (1886), and Mall (1887) before it was correctly identified by Kastschenko (1887). The sixth pouch was



overlooked until Kallius (1905) and Rabl (1907b) discovered it in the duck (*Anas platyrhynchos*). Subsequently, Grosser and Tandler (1909) and Sicher (1921) found it in the lapwing (*Vanellus vanellus*), and Johnson (1918a, 1918b) reported its presence in embryos of the chicken, the pied-billed grebe (*Podilymbus podiceps*), the gallinule (*Gallinula chloropus galeata*), the coot (*Fulica americana*), and Forster's tern (*Sterna forsteri*).

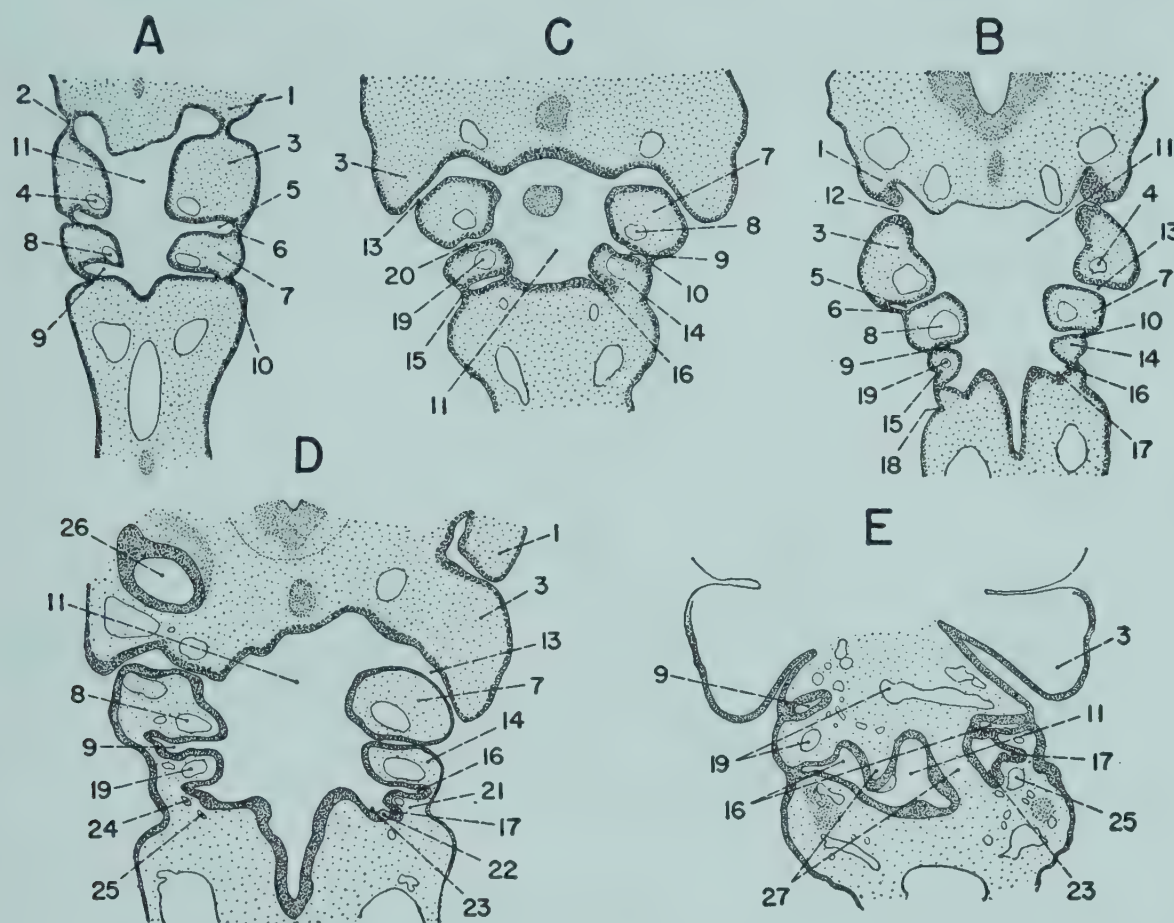
Studies of the chicken, the zebra parakeet (*Melopsittacus undulatus*), and the lapwing (*Vanellus vanellus*) indicate that the visceral pouches appear in succession between the stages of 11 and 48 somites. In embryos of these species, the development of the pharyngeal region proceeds at a similar rate, since it is always at a more or less equivalent stage in embryos of the same somite number (Keibel and Abraham, 1900; Abraham, 1901; Grosser and Tandler, 1909).

Only the first three pairs of visceral pouches become perforated (Kastschenko, 1887; Liessner, 1887). The most anterior pair evaginates when there are 11 to 14 somites, or during the latter half of the chick's second incubation day. The first pair of visceral pouches are merely localized prolongations of the dorsolateral arms of the cephalic fore-gut, which, at this time, is crescentic in cross section (cf. Fig. 171). At the level of the heart, the fore-gut exhibits a median ventral evagination (Duval, 1889; Livini, 1903) which forms a longitudinal groove, called the meso-branchial groove (Kallius, 1905). After another pair of somites has been added, the outer end of the first pouch on each side comes in contact with the ectoderm of the first visceral furrow. The latter is a shallow groove, transverse to the long axis of the embryo, on the ventrolateral body wall approximately at the level of the ectodermal head fold and immediately anterior to the already slightly projecting second visceral arch (see Fig. 359-A in Chapter 12). The first furrow, like the second and third, also possesses a dorsal component in the form of a pitlike depression situated higher on the lateral body wall than the groove. The first, second, and third visceral furrows are not directly opposite their corresponding visceral pouches, but somewhat posterior to them. Consequently, contact is not between the apex of a pouch and the deepest part of a furrow, but rather between the anterior face of the furrow and the posterior face of the pouch (Fig. 173-A and B). The thin membrane formed by the apposed ectoderm and endoderm slants forward and outward and therefore is not parallel but almost perpendicular to the embryo's long axis (Kastschenko, 1887).

The second visceral pouch forms almost as soon as the first pouch and first furrow meet—that is, at 12- to 15-somite stages—and in turn makes contact with the second visceral furrow at stages of 17 to 19 somites. The second furrow is initially somewhat shorter than the first and lies directly



behind the otic pit. The third pair of endodermal pouches and ectodermal furrows appears behind the second, either very late in the chick's second incubation day or very early in the third day, that is, within the period when the nineteenth to twenty-third somites are forming. Figure 174-A shows the chick's fore-gut *in situ* at this time. (The third visceral pouch and furrow are sometimes termed the first branchial pouch and furrow.)



**Fig. 173.** Cross sections through the pharyngeal region of duck (*Anas platyrhynchos*) and chick (*Gallus gallus*) embryos, at various stages of development. (Redrawn with modifications A, after Mall, 1887; B and C, after Liessner, 1887; D and E, after Rabl, 1907b.)

A, from a 70-hour chick embryo; B, from a 90-hour chick embryo; C, from a 108-hour chick embryo; D, from a 113-hour duck embryo; E, from a 119-hour duck embryo. All  $\times 20$ .

1, first visceral arch; 2, first visceral furrow; 3, second visceral arch; 4, second aortic arch; 5, second visceral pouch; 6, second visceral furrow; 7, third visceral arch; 8, third aortic arch; 9, third visceral pouch; 10, third visceral furrow; 11, pharynx; 12, first visceral cleft; 13, second visceral cleft; 14, fourth visceral arch; 15, fourth visceral furrow; 16, fourth visceral pouch; 17, fifth visceral pouch; 18, fifth visceral furrow; 19, fourth aortic arch; 20, third visceral cleft; 21, fifth visceral arch; 22, sixth visceral arch; 23, sixth visceral pouch; 24, fifth aortic arch; 25, sixth aortic arch; 26, otic vesicle; 27, arytenoid processes.

The mesobranchial groove now disappears anteriorly, leaving as its only remnant an epithelial thickening which is the first visible primordium of the thyroid gland (*Livini, 1903*). This primordium lies immediately anterior to the second visceral arch (*Kastschenko, 1887; Kallius, 1905*). Posteriorly, the mesobranchial groove is continued caudad and is found at the level of the third visceral arch and throughout the postbranchial



pharynx. It may now be called the laryngotracheal groove, since the larynx and trachea develop from it (Kastschenko, 1887; Locy and Larsell, 1916a). The anterior end of the laryngotracheal groove is flanked by the bilateral arytenoid processes, bulging mesodermal masses which participate in

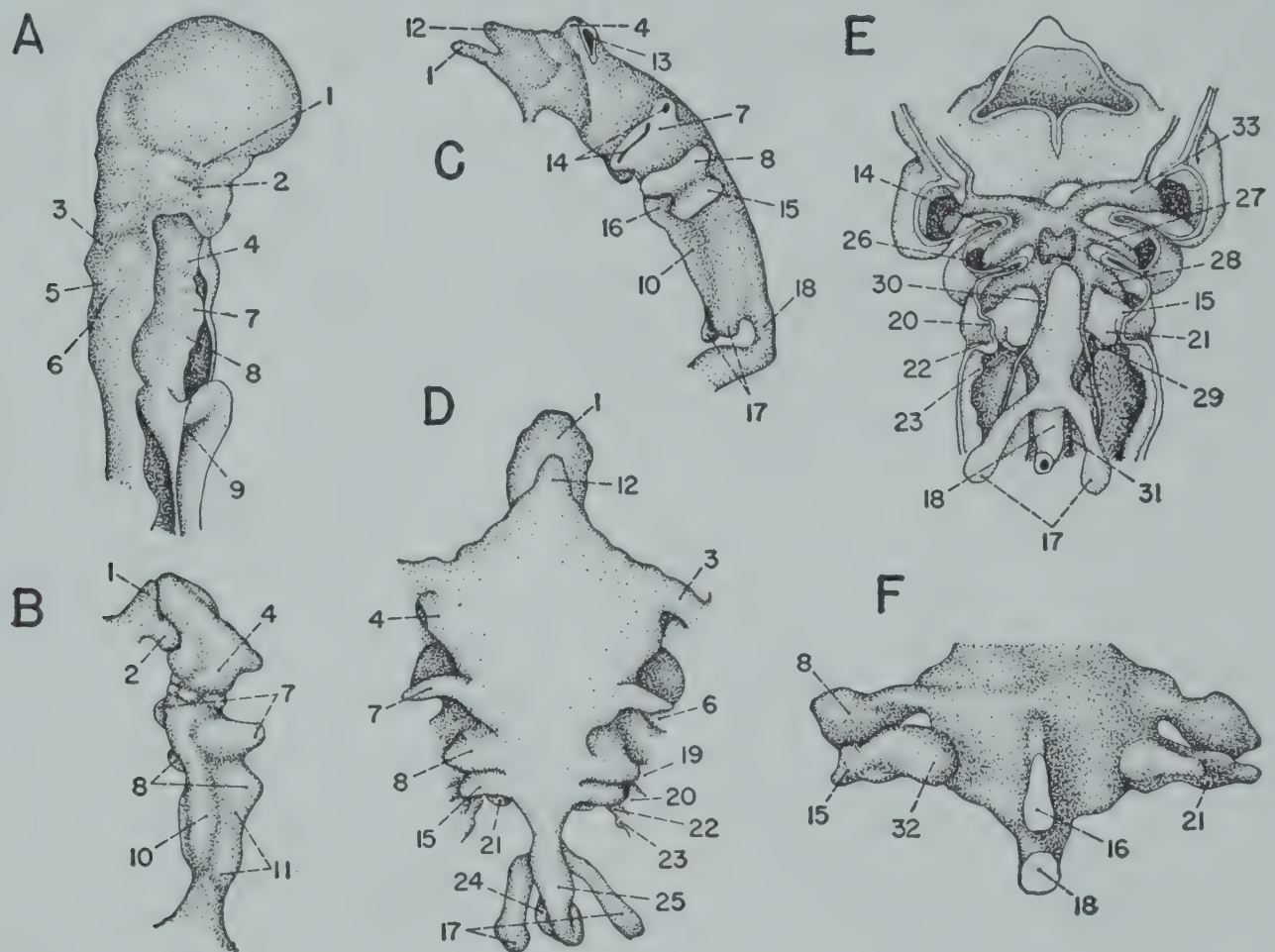


Fig. 174. External views of the developing pharynx in chick (*Gallus gallus*) and duck (*Anas platyrhynchos*) embryos. (Redrawn with modifications A to E, after Kastschenko, 1887; F, after Rabl, 1907b.)

A, the pharynx of the chick embryo at the end of the second incubation day, seen *in situ* from the right ventral side after removal of the ventral ectoderm; B, the isolated fore-gut of the chick at the beginning of the third incubation day, seen from the left ventral side; C, isolated pharynx from a 3-day chick embryo, seen from the left side; D, dorsal view of chick pharynx at the beginning of the fourth incubation day, blood vessels not shown; E, ventral view of same, with blood vessels included; F, ventral view of the posterior part of the pharynx from a 5-day duck embryo. All  $\times 15$ .

1, Rathke's pouch; 2, ectodermal oral invagination; 3, first visceral furrow; 4, first visceral pouch; 5, otic invagination; 6, second visceral furrow; 7, second visceral pouch; 8, third visceral pouch; 9, anterior intestinal portal; 10, developing trachea; 11, respiratory portion of pharynx; 12, Seessel's pouch; 13, first visceral cleft; 14, second visceral cleft; 15, fourth visceral pouch; 16, primordium of larynx; 17, primordia of lungs; 18, esophagus; 19, third visceral furrow; 20, fourth visceral furrow; 21, fifth visceral pouch; 22, fifth visceral furrow; 23, sixth visceral furrow; 24, stomach; 25, crop; 26, third visceral cleft; 27, third aortic arch; 28, fourth aortic arch; 29, sixth aortic arch; 30, pulmonary artery; 31, aorta; 32, sixth visceral pouch; 33, second aortic arch.

larynx formation. The laryngotracheal groove apparently does not exist in the sparrow (*Passer domesticus*), the martin (*Delichon urbica*), or the goose (*Anser anser*), or at least not before the lung primordia differentiate (Rösler, 1911). Figure 174-B shows the chick's fore-gut as it would



appear if removed from the body at the beginning of the third day. The laryngotracheal groove can be seen projecting as an external ridge. It will be noted that the visceral pouches are much larger than at the 2-day stage.

While the twenty-fifth to thirtieth somites are being added, the fourth visceral (second branchial) pouch evaginates, the third comes into contact with the ectoderm, and the lung primordia appear; in the chick, these events take place during the first half of the third incubation day. The earliest indication of lung development is a bilateral expansion of the laryngotracheal groove at its caudal end, immediately posterior to the fourth visceral pouch. The two shallow lung pouches forming this expansion grow out in a caudolateral and slightly dorsal direction, and their divergent distal ends have separated from the esophagus by the sixtieth hour (*Locy and Larsell, 1916a*).

The first two visceral pouches become clefts, or fissures, in the course of the chick's third day when perforations form in their occluding membranes of ectoderm and endoderm. The first pouch breaks through to the outside only in its dorsal area of contact with the first visceral furrow, because the slitlike ventral part of the furrow has become compressed between the massive first and second visceral arches and has been transformed into an epithelial plate (*Kastschenko, 1887*). The second pouch, however, opens both dorsally and ventrally, and thus the second cleft has two divisions.

By the end of the chick's third day, the paired lung primordia have grown out as distinct ventrolateral diverticula which project caudad at the posterior end of the postbranchial pharynx. The thyroid anlage has begun to evaginate. In the duck (*Anas platyrhynchos*) embryo of a more or less equivalent developmental stage (42 somites), there is a median elevation in the floor of the pharynx immediately behind the thyroid evagination, at the level of the second visceral arch; this elevation is the copula of the second arch (*Kallius, 1905*). The entire pharyngeal gut of the 3-day chick embryo is shown in isolation in Fig. 174-C. Two more weak visceral arches and furrows have been added behind the third arch, which is now somewhat less prominent than previously (*Boyden, 1918*).

Either at the end of the chick's third day or early in the fourth day, the third visceral pouch becomes perforated, the fourth contacts the ectoderm, and the fifth appears. The fifth pouch has been observed in zebra parakeet (*Melopsittacus undulatus*), duck (*Anas platyrhynchos*), and lapwing (*Vanellus vanellus*) embryos of 34 to 43 somites (*Abraham, 1901; Rabl, 1907b; Grosser and Tandler, 1909*), in the 46-hour grebe (*Podilymbus podiceps*) embryo (*Johnson, 1918a*), and in gallinule (*Gallinula chloropus galeata*) and Forster's tern (*Sterna forsteri*) embryos incubated 4.5 days (*Johnson, 1918b*). As Fig. 173-D shows, it is essentially an



evagination of the posterior wall of the fourth pouch (*Kastschenko*, 1887; *Rabl*, 1907b). In the chick, the sixth pouch forms a few hours after the fifth (cf. Fig. 173-D); it appears in the grebe in the middle of the fifth day (*Johnson*, 1918a) and in the lapwing about at the 48-somite stage (*Grosser and Tandler*, 1909). The sixth pouch is a second evagination of the posterior wall of the fourth pouch and is medial to the fifth pouch, which is now directed caudolaterally rather than caudally. A wide zone of mesoderm intervenes between the fifth and sixth pouches and the ectoderm, with which neither of these pouches ever comes in contact. Both the fifth and the sixth pouches are formed of high cylindrical epithelium (*Rabl*, 1907b; *Dudley*, 1942) and are smaller than the others—in the 4.5- to 5-day duck (*Anas platyrhynchos*) embryo, the third pouch measures 0.32 mm. dorsoventrally and the fourth 0.26 mm., whereas the newly differentiated fifth and sixth pouches are only 0.19 mm. and 0.1 mm. long, respectively. An internal ridge of tissue, containing the fifth aortic arch, projects into the pharyngeal cavity between the fourth and fifth visceral pouches, and a similar ridge, bearing the sixth aortic arch, is found between the fifth and sixth pouches; these ridges have been interpreted as rudimentary fifth and sixth visceral arches (*Rabl*, 1907b).

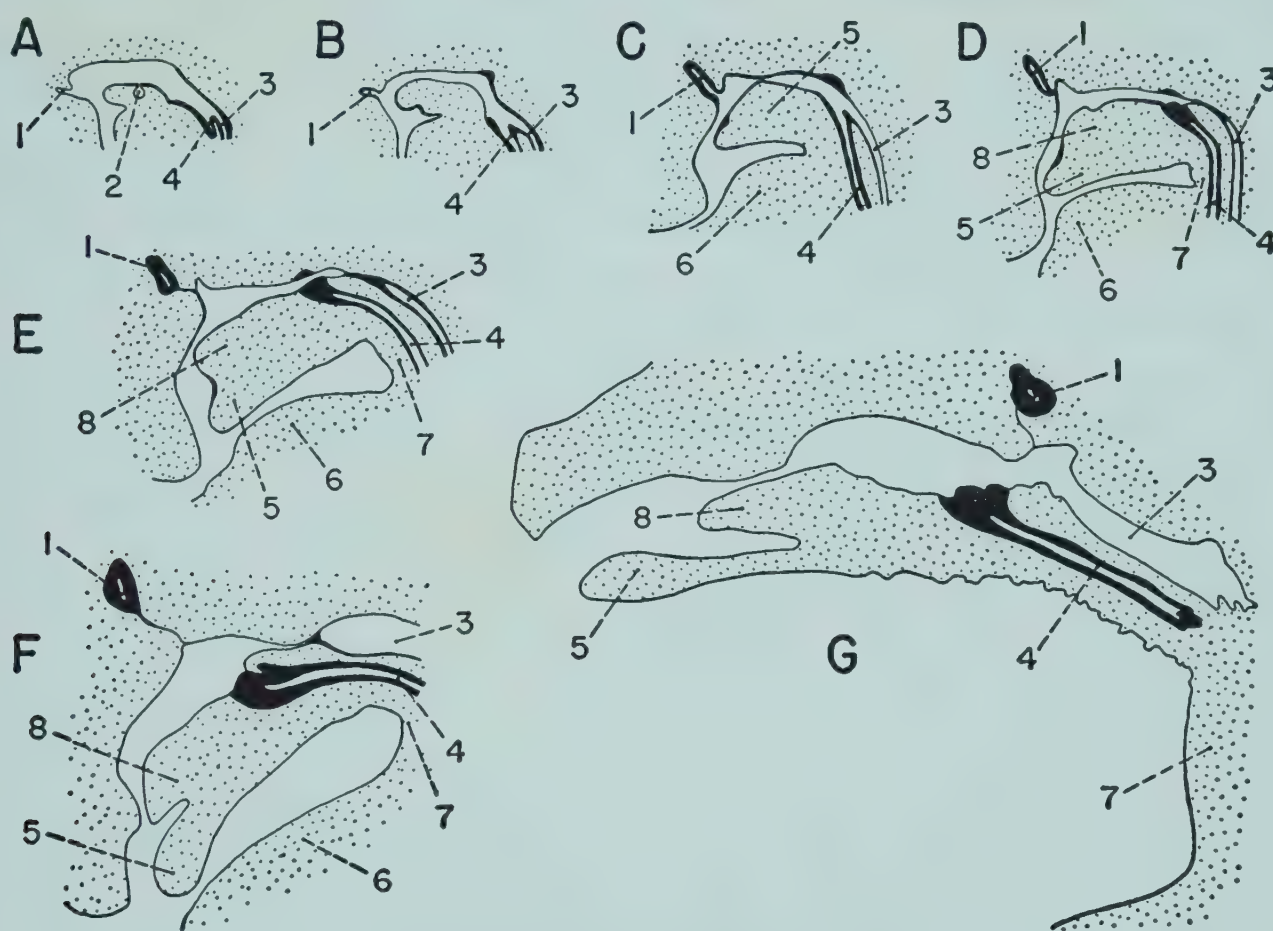
The visceral pouches and clefts are at the height of their development at the beginning of the chick's fourth day of incubation (*Kastschenko*, 1887). Dorsal and ventral views of the isolated pharyngeal portion of the fore-gut, as it appears at this time, are given in Fig. 174-D and E. Figures 175-A and B show sagittal sections of the canary (*Serinus canaria*) embryo's pharynx at stages corresponding to those reached by the chick after 3 to 4 days of incubation. The advance in the evolution of the respiratory tract is to be noted. It may be seen that the pharynx is narrower ventrally than dorsally just behind the fifth pouches. Here the bilateral arytenoid processes project into the pharyngeal cavity from the ventral side. At this level, these processes soon span the lateral parts of the pharyngeal cavity from dorsal to ventral (cf. Fig. 173-E). They then extend farther craniad, thus reducing the size of the communication between the pharynx and the fourth, fifth, and sixth pouches. Figure 174-F, which represents the exterior of the 5.5-day duck (*Anas platyrhynchos*) embryo's pharynx in postero-ventral view, shows how the sixth pouches are separated from the slitlike lumen of the trachea (*Rabl*, 1907b).

During the chick's fourth day, the pharyngeal region enters upon a period of change that eventually results in the disappearance of the visceral pouches, except for certain remnants which are transformed into other structures. The pitlike dorsal openings of the first and second clefts close before the end of the fourth day. By this time, the ventral part of the first pouch has become separated from the ectoderm, and this pouch can now be regarded as the primitive tubotympanic cavity. The fifth furrow has



disappeared (*Kastschenko*, 1887). The thyroid primordium is connected with the pharynx of a pedicle (*Mall*, 1887).

The third visceral cleft closes during the chick's fifth day (*Meuron*, 1886b; *Kastschenko*, 1887; *Liessner*, 1887). Before the middle of the fifth day, the thyroid primordium separates from the floor of the pharynx (*Meuron*, 1886b). By the end of the same day, the caudad growth of the second arch has greatly elongated the second visceral furrow. Meantime, the lateral growth of the third and fourth visceral arches has stretched out the medial portions of the third and fourth pouches so that they are now



**Fig. 175.** Diagrammatic representations of sagittal sections through the region of the oral cavity and pharynx of the canary (*Serinus canaria*) embryo. (Redrawn with modifications after *Stellwaag*, 1912.)

A to G show successive stages equivalent to those seen in the chick embryo between the third or fourth day and the twelfth or thirteenth day. All  $\times 8$ .

1, hypophysis; 2, thyroid; 3, esophagus; 4, trachea; 5, lower jaw; 6, thorax; 7, neck; 8, tongue.

long, narrow endodermal tubes (endobranchial ducts) connecting the distal, larger portions of the pouches to the pharynx (*Meuron*, 1886b; *Kastschenko*, 1887; *Mall*, 1887; *Dudley*, 1942). Growth of the third and fourth arches also results in some invagination of the ectoderm of the third and fourth furrows to form similar ectodermal tubes (ectobranchial ducts) connecting the pouches with the surface epithelium.

In the chick, the ectobranchial and endobranchial ducts become solid, lose their respective connections with ectoderm and endoderm, and atrophy at varying times between the end of the fifth day and the end of



the sixth (Meuron, 1886b; Kastschenko, 1887; Mall, 1887; Verdun, 1898; Dudley, 1942; Schrier and Hamilton, 1952); in the grebe (*Podilymbus podiceps*), their disappearance occurs about a day later (Johnson, 1918a). Rupture of these structures is largely the result of strain exerted upon them as they are pulled backward by the third and fourth aortic arches. The latter lie directly anterior to the third and fourth visceral pouches, respectively, and are migrating caudad during this period (Kastschenko, 1887; Rabl, 1907b; Dudley, 1942). Simultaneously, there is an actual and relative anteroposterior shortening of the pharyngeal cavity at and posterior to the level of the third pouch, so that the glottis migrates forward (Kallius, 1905). Sagittal sections of equivalent stages in the canary (*Serinus canaria*) embryo may be seen in Fig. 175-C, D, and E.

The ectodermal tissue of the second visceral furrow, which has gradually lost its lumen, breaks up and atrophies after the chick's sixth day (Kastschenko, 1887). The second pouch has entirely disappeared by the seventh day (Bemmelen, 1886). The third pouch gives rise to thymus and parathyroid tissue (see Chapter 11) according to observers of the chicken (Verdun, 1898; Schrier and Hamilton, 1952), the duck, *Anas platyrhynchos* (Hamilton, 1913), the sparrow, *Passer domesticus* (Helgesson, 1913), the grebe, *Podilymbus podiceps* (Johnson, 1918a), and the lapwing, *Vanellus vanellus* (Sicher, 1921). Failure to recognize the fifth and sixth pouches has led to confusion regarding the derivatives of the fourth pouch, but it appears fairly probable that this pouch makes exactly the same contribution as the third (Verdun, 1898; Dudley, 1942; Schrier and Hamilton, 1952); that the fifth pouch disappears (Rabl, 1907b; Johnson, 1918b; Dudley, 1942), or possibly becomes incorporated into the fourth (Johnson, 1918a); and that the sixth pouch is the primordium of the ultimobranchial or postbranchial body (Rabl, 1907b; Johnson, 1918a; Sicher, 1921; Dudley, 1942), an organ of uncertain function (Dudley, 1942). This body becomes independent of the branchial endoderm on the chick's sixth day (Terni, 1927).

## THE ORAL CAVITY

Only a portion of the tissue lining the mouth cavity is derived from the embryonic fore-gut. The remainder develops from the stomodaeum, a depression on the ventral surface of the head formed passively by the outgrowth of the ectoderm-covered mesoderm of the head and the first visceral arch around the oral plate or pharyngeal membrane. When the oral plate ruptures, the ectoderm-lined stomodaeum becomes confluent with the lumen of the fore-gut and is thus added to the endodermal gut tube as the latter's most anterior segment.

As previously mentioned, the oral plate lies at first in the ventral midline



practically at the tip of the head. The neural tube grows out beyond the fore-gut between the 6- and 9-somite stages (cf. Fig. 171-D) and has begun to bend ventrally in front of the latter by the 15-somite stage (cf. Fig. 171-E) in both the duck, *Anas platyrhynchos* (Rex, 1897), and the chick (Salvi, 1903; Adelmann, 1922). This flexure of the neural axis causes a downward curvature of the notochord and the prechordal plate of mesoderm and initiates the separation of the prechordal mesoderm from the neural tube and from the anterior end of the fore-gut roof (Fig. 176-A). The subsequent increase in the cranial flexure sharply indents the ectoderm on the ventral surface of the head at the point where the prechordal plate terminates (Fig. 176-B), that is, just anterior to the pharyngeal membrane (Salvi, 1903; Adelmann, 1922). The ectoderm-lined pocket that is thus produced between the overhanging forebrain and the oral plate is the primordium of the pars buccalis of the hypophysis and is known as Rathke's

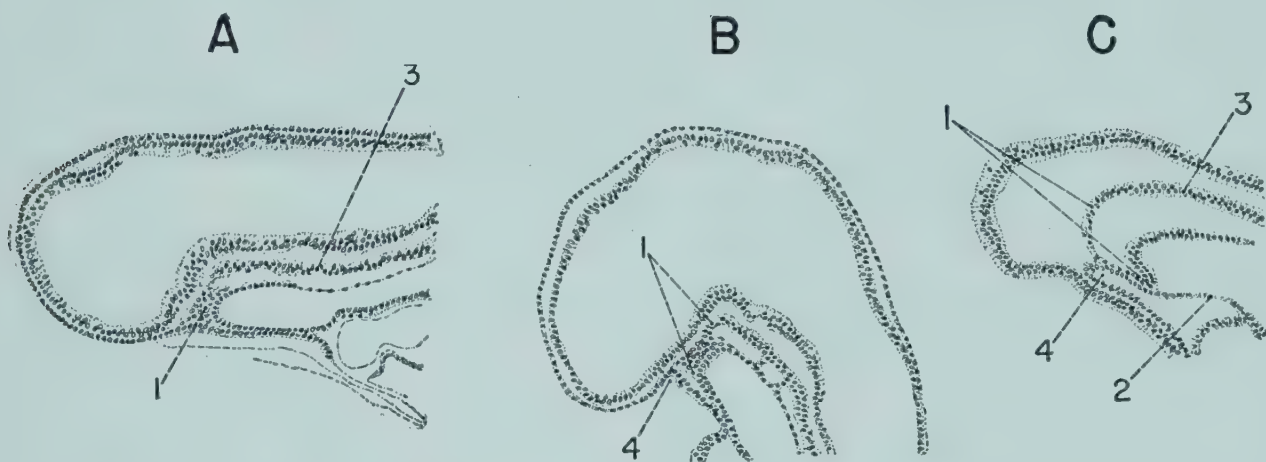


Fig. 176. Stages in the development of the oral portion of the fore-gut in the chick embryo, as shown by midsagittal sections. (Redrawn after Adelmann, 1922.)

A, 15-somite stage; B, 19-somite stage; C, 29- to 30-somite stage. All  $\times 35$ .

1, prechordal plate; 2, oral membrane; 3, notochord; 4, oral hypophysis.

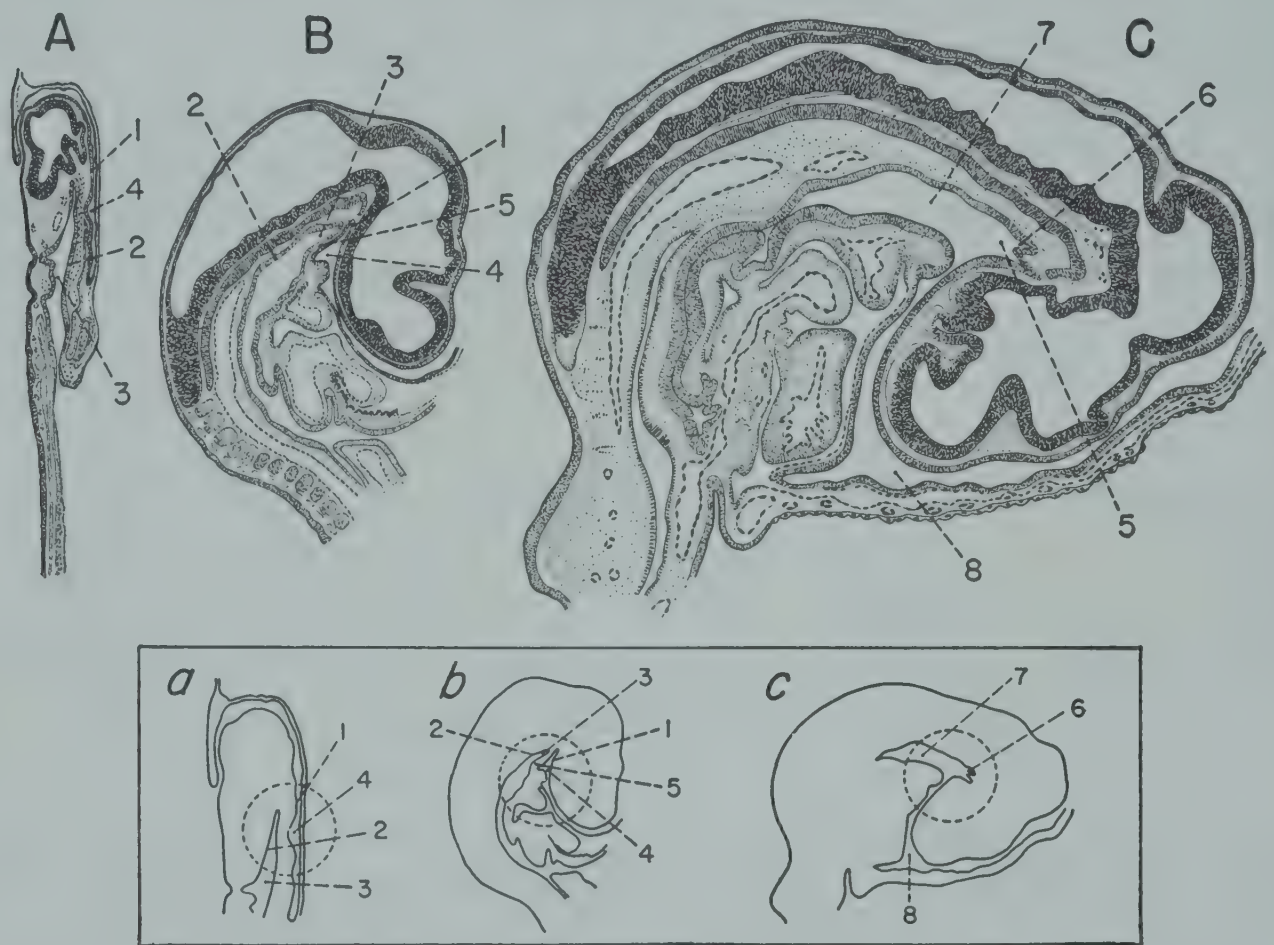
pouch (Fig. 176-C). Figure 177 shows the relationship of the developing oral cavity to other structures present in the young embryo.

According to Rex (1897), the central part of the oral part has begun to thin out in the 16-somite duck (*Anas platyrhynchos*) embryo. Manno (1903) noted the incipient degeneration of the outer, ectodermal component of the membrane in the 61-hour chick embryo and its complete absence in the 72-hour embryo (Fig. 178-A, B, and C); Nicolas and Weber (1901) reported the same condition in the duck (*Anas platyrhynchos*) embryo incubated 72 hours. Almost immediately after the destruction of the ectodermal portion of the membrane, the endodermal layer becomes perforated (Fig. 178-D and E). The latter disappears entirely within the chick's next 7 or 8 hours of incubation (Manno, 1903). Rupture of the oral plate has been observed at the 26- to 27-somite stage in the European lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909); at the 29-somite stage in the duck, *Anas platyrhynchos* (Rex, 1897); at 28- to 34-somite



stages in the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901); and at 31- to 38-somite stages in the chicken (Keibel and Abraham, 1900).

Before the oral plate disappears, the anterior end of the fore-gut has been prolonged forward somewhat as a "blindly ending pocket" known as the preoral gut, or Seessel's pouch, which is approximately coextensive with Rathke's pouch. The prolongation of the fore-gut is apparently the result of the adherence of the anterior end of its roof to the prechordal mesoderm. As the head bend becomes sharper, the posterior end of the prechordal



**Fig. 177.** Stages in the development of the oral cavity, showing its relationship to other early embryonic structures. (Redrawn with rearrangement after Duval, 1889.)

A, anterior portion of the chick embryo, in sagittal section, 43-hour stage; B, the same, 52-hour stage; C, the same, 82-hour stage. All  $\times 13$ .

*Insert:* a, b, and c, shown diagrammatically, with the region of the oral cavity encircled.

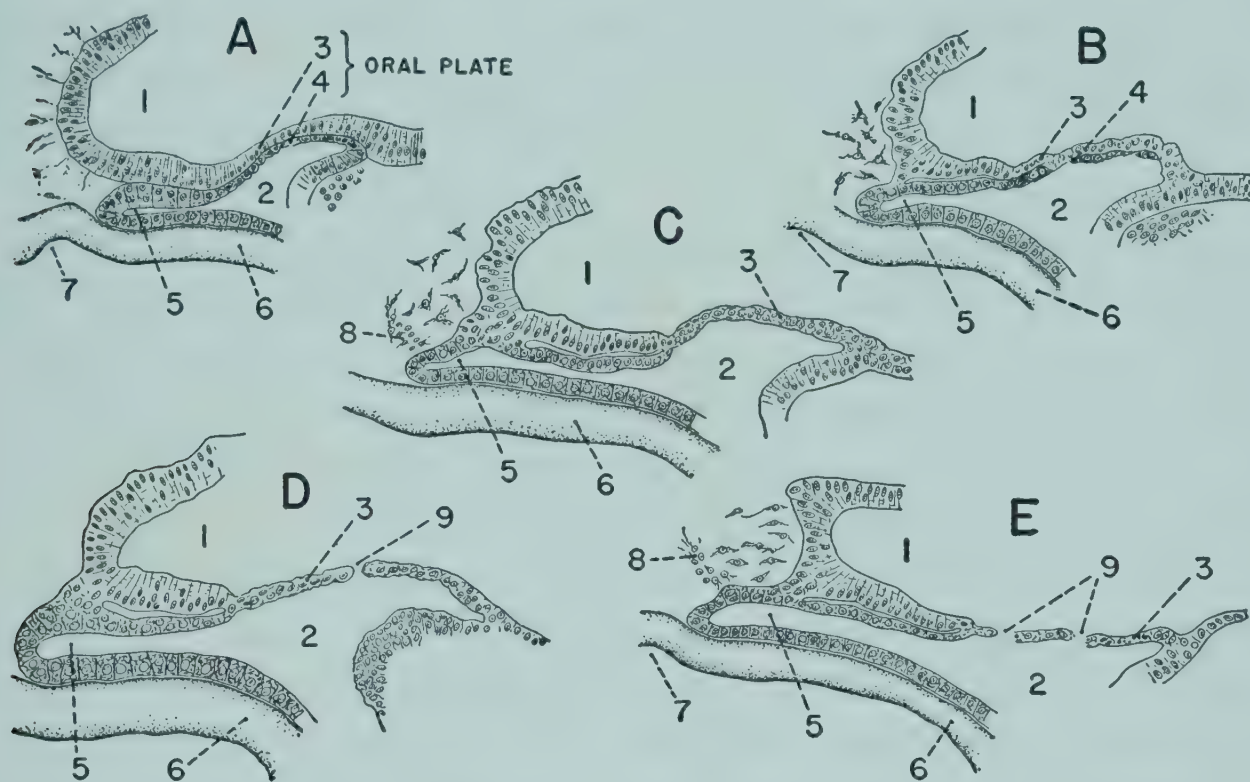
1, ectoderm; 2, endoderm; 3, fore-gut; 4, stomadaeum; 5, oral plate (or its former site); 6, Seessel's pouch; 7, pharynx; 8, amniotic cavity.

plate rotates forward around its anterior end. The detachment of the gut endoderm from the prechordal mesoderm is not sufficiently rapid to prevent the most cranial part of the fore-gut from being carried forward (cf. Fig. 176-C). After the rupture of the oral plate, the dorsal wall of the preoral gut probably collapses against its ventral wall to produce a solid knob of tissue, visible in the 6-day chick or duck (*Anas platyrhynchos*) embryo (Saint-Remy, 1895) behind the hypophyseal stalk (Seessel, 1877) or applied against it (Bawden, 1893). This remnant of Seessel's pouch eventually disappears, apparently without participating in the formation of the hypophysis.



After the oral plate degenerates, Rathke's pouch appears as an evagination of the dorsal wall of the oral cavity. The developing hypophysis constitutes a landmark indicating the posterior extent of the ectoderm on the roof of the mouth, since Rathke's pouch originally lies at the inner end of the stomodaeum.

The stomodaeum is first recognizable near the end of the chick's second incubation day (Mall, 1887; Moldenhauer, 1877; Duval, 1889) when the maxillary and mandibular processes of the first visceral arch begin to project



**Fig. 178.** Successive stages in the perforation of the oral plate of the chick embryo. (Redrawn with modifications after Manno, 1903.)

A, at 61 hours, oral plate composed of inner (endodermal) and outer (ectodermal) layer; B, at 68 hours, degeneration of outer layer beginning; C, at 72 hours, oral plate composed of endoderm only, degeneration of ectodermal layer complete; D, at 74 hours, initial perforation apparent in endodermal layer; E, at 75 hours, many perforations in endodermal layer, which is soon to disintegrate and disappear. All  $\times 50$ .

1, anterior extremity of the fore-gut (Seessel's pouch); 2, stomodaeum; 3, endodermal component of the oral plate; 4, ectodermal component of the oral plate; 5, Rathke's pouch; 6, wall of brain; 7, infundibulum; 8, anterior end of notochord; 9, perforations in the endoderm of the oral plate.

ventrally on either side of the oral plate. At this time, the ventral surface of the overhanging forebrain region of the head forms the roof of the stomodaeum. During the first half of the third day, the cranial flexure increases so that the ventral surface of the head faces dorsally and is opposite and parallel to the oral plate; Rathke's pouch is greatly flattened (cf. Fig. 176-C; Fig. 178). Simultaneously, the maxillary and mandibular processes grow out farther. By the end of the third day, the angle between the forebrain and the hindbrain is no longer acute, but about 90 degrees, and the ventral surface of the head is parallel to the axes of the now con-



siderably more prominent maxillary and mandibular processes (*Born, 1879; Mall, 1887; Duval, 1889*).

At the end of the chick's fourth day, the stomodaeum, viewed from the ventral side, is bounded by the bilateral maxillary and mandibular processes and by the projecting head (see Fig. 166 in Chapter 5) and thus appears to have an irregular five-sided outline (*Goette, 1867*). At this time (*Mall, 1887*) or in the middle of the fifth day (*Duval, 1889*), the frontal process begins to bulge slightly between the nasal fossae.

At the 4.5-day stage, the palatine process can be seen as a ridge along the medial surface of each maxillary process. Thin extensions of the palatine processes start to grow medialward on the eighth day; on the eleventh day, they meet in the midline without fusing (*Born, 1879*) and thus form the cleft palate which separates the oral cavity from the nasal cavity.

Meantime, the upper and lower beaks develop. By the end of the fifth day (*Goette, 1867; Mall, 1887*), the fused mandibular processes begin to grow out, especially in the midline, to form the lower beak. The upper beak, which consists of the fused frontal, maxillary, and nasal processes (see Chapter 5), grows forward much more rapidly than the lower beak. The floor of the oral cavity, therefore, does not extend itself forward as quickly as the roof until some time after the ninth or tenth day of development, when the lower beak becomes as long as the upper. It is of interest to note that *Kuo (1932a, 1932b)* observed spontaneous opening and closing of the mouth, or beak, at all times after the fifth or sixth day of incubation. By the ninth day, the frequency of beak movements has increased from two or three per minute to as many as eleven per minute. It drops to about four per minute as the yolk sac grows over the embryo's head, but again rises slightly (to six per minute) after the sixteenth or seventeenth day, when the head is once more uncovered.

A feature of the later stages in the development of the oral cavity is a widening of the angle that it forms with the esophagus and trachea (cf. Fig. 175).

### The Tongue

In birds, the tongue chiefly serves the function of prehension and varies greatly in shape in accordance with the diversified feeding habits of different species. It is supported by the hyoid apparatus (whose development is described in Chapter 12) and is moved by several muscles attached to the various bones of this apparatus and to the mandibles. The surface of the tongue is horny but is provided with glands.

The ventral portions of the first three visceral arches contribute the material which forms the tongue (*Reichert, 1837; Mall, 1887; Kallius, 1905*). The greater part of its substance develops from thickenings of the mesoderm beneath the floor of the embryonic pharynx. Its epithelial cover-



ing, therefore, is endodermal in origin, except for a small portion derived from the ectoderm of the mandibular process just anterior to the oral plate.

Kallius (1905) made a thorough study of the development of the tongue in the duck (*Anas platyrhynchos*) and the sparrow (*Passer domesticus*). The following account is based largely upon his findings.

### *The External Shape of the Tongue*

The major primordium of the tongue, the tuberculum impar, is a median elevation that appears on the floor of the pharyngeal cavity soon after the formation of the thyroid evagination. In the duck (*Anas platyrhynchos*), the thyroid anlage is seen at the 42-somite stage in the ventral midline of the pharyngeal floor, at the level of the first visceral pouches. Directly behind the thyroid primordium, at the level of the second visceral arches, there is a bulging area shaped like a triangle with apex directed posteriorly. The first visceral arches now grow forward somewhat, and the tuberculum impar differentiates immediately anterior to the thyroid evagination (Fig. 179-A); Kallius (1903) found it in the same location in the chick and zebra parakeet (*Melopsittacus undulatus*). It is to be assumed that the tuberculum impar, at the time of its appearance, is located at the first visceral arches, although the first visceral pouches no longer extend to the floor of the pharynx and the boundary between the first and second pairs of arches is therefore indefinite. According to Kallius (1905), the tuberculum impar arises posterior to the site of the now vanished pharyngeal membrane and hence is covered with endoderm.

Very soon the tuberculum impar elongates until it obviously extends into the territory of the second visceral arch. The thyroid primordium, apparently displaced by the growth of the tuberculum impar, is now found at the level of the second visceral pouch, and the third arches are separated ventrally by an elevation that seems to correspond to the elevation formerly present between the second arches.

The mandibular processes, which are separated anteriorly by a notch in the ventral midline, continue to grow forward. The median notch is bordered by a low ridge, behind which there is a V-shaped groove parallel to the ridge (cf. Fig. 175-D). Lateral to the arms of the groove, the first visceral arches begin to bulge upward into the oral cavity. These bilateral bulging areas are the lateral lingual ridges (Fig. 179-B). Kallius (1905) believed that they are situated anterior to the level of the oral plate and hence are invested with ectoderm.

With the further growth of the mandibular processes, the tuberculum impar enlarges and becomes fused anterolaterally with the lateral lingual ridges. A median groove begins to appear in the tuberculum impar. Anteriorly, a median ridge, the primordium of the frenulum linguae, connects the tuberculum impar with the low ridge bordering the notch between the



mandibular processes (Fig. 179-C). The V-shaped groove is thus transformed into two grooves diverging from each other anteriorly at an angle of about  $90^\circ$ . The forward growth of the floor of the mouth carries the ventral ends of the third visceral arches anteriorly. The glottis is also migrating craniad. It is about at this time that the precartilaginous anlagen of the hyobranchial skeleton begin to appear (see Chapter 12).

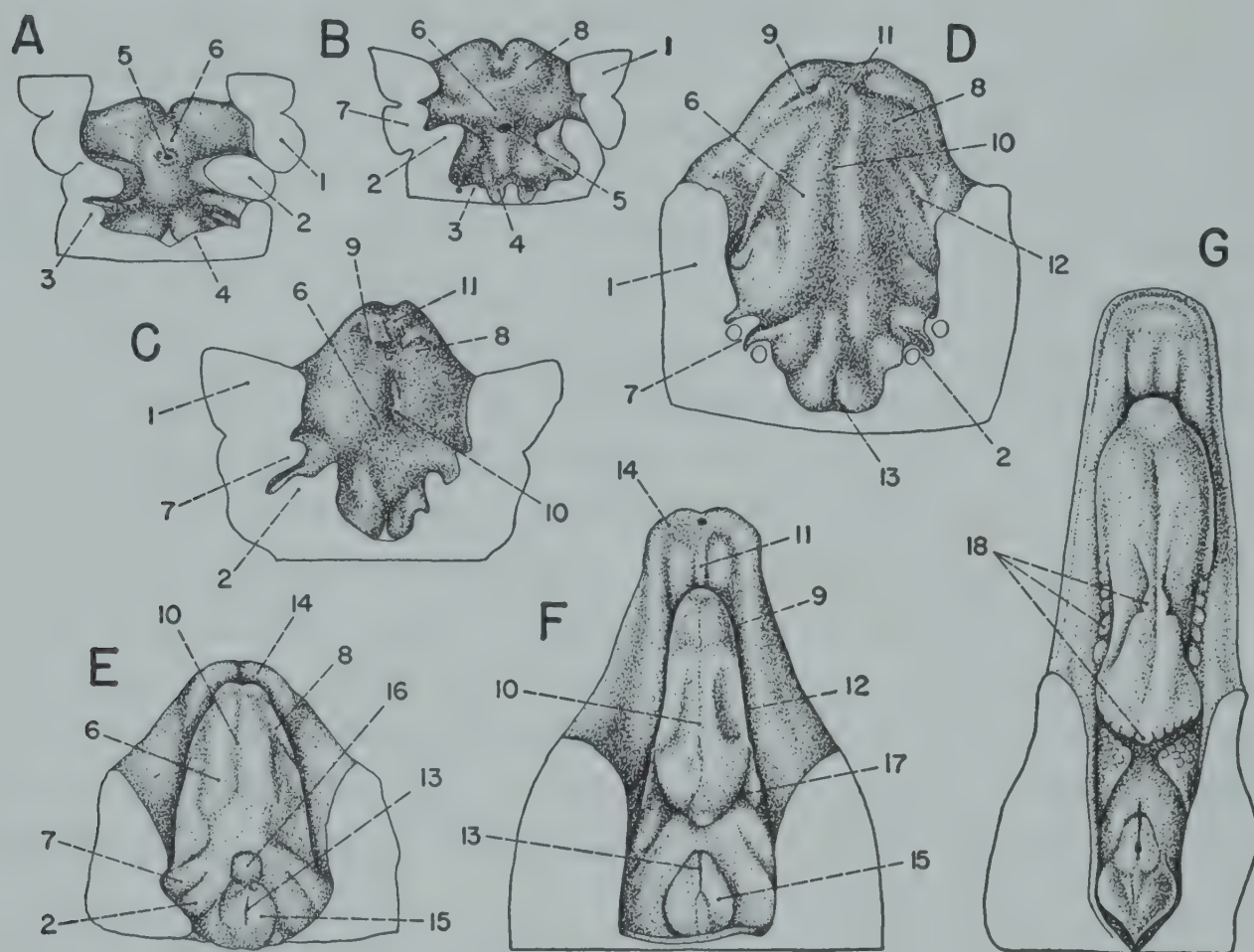


Fig. 179. Stages in the development of the tongue in the embryo of the duck (*Anas platyrhynchos*), as seen after removal of the roof of the pharyngeal region. (Redrawn with modifications after Kallius, 1905.)

A, from a 7.5-mm. embryo ( $\times 12$ ); B, from an 8.0-mm. embryo ( $\times 12$ ); C, from a 10.5-mm. embryo ( $\times 12$ ); D, from a 12.25-mm. embryo ( $\times 12$ ); E, from a 15.0-mm. embryo ( $\times 4$ ); F, from a 22.0-mm. embryo ( $\times 4$ ); G, from an embryo with a head length of 24.0 mm. ( $\times 4$ ).

1, first visceral arch; 2, third visceral arch; 3, fourth visceral arch; 4, sixth visceral arch; 5, thyroid primordium; 6, tuberculum impar; 7, second visceral arch; 8, lateral lingual ridge; 9, anterior limiting furrow; 10, median groove; 11, frenulum; 12, lateral lingual furrow; 13, glottis; 14, lower jaw; 15, arytenoid ridges; 16, rudimentary epiglottis; 17, posterior limiting furrow; 18, papillae.

Soon the region corresponding to the first and second visceral arches is six times as long as the rest of the pharynx. The lower jaw has grown forward markedly, especially in the midline. So great has been its prolongation that its median notch has almost disappeared and the ridges and grooves formerly bordering the notch are not only longer but are also reversed in direction; that is, they now diverge from each other posteriorly instead of anteriorly (Fig. 179-D). The grooves indicate the anterior limits



of the definitive tongue. Directly behind the grooves are the lateral lingual ridges, which form the anterolateral edges of the tongue. The lateral lingual ridges are separated from the tuberculum impar by two shallow longitudinal furrows (the lateral lingual furrows); the latter are continuous posteriorly with the grooves that are the remnants of the first visceral pouches. The tuberculum impar is now traversed from end to end by a median furrow. Behind the tuberculum impar, at the level of the third visceral arch, the median elevation is demarcated laterally by a slight groove on each side. Immediately posterior to the median elevation is the slitlike opening into the trachea; this opening is now still farther forward than previously.

The lateral lingual furrows soon become continuous with the anterior limiting furrows, so that the lateral lingual ridges are no longer distinguishable from the tuberculum impar. Two longitudinal ridges, each delimited laterally by a shallow furrow, now flank the median groove. The latter does not extend back as far as formerly; therefore, the posterior end of the tuberculum impar is now flat. The median elevation behind the tuberculum impar has become a well-demarcated rectangular area and is recognizable as a rudimentary epiglottis (Fig. 179-E). The slitlike glottis lies between the arytenoid ridges, which form an oval protruding area.

In the duck (*Anas platyrhynchos*) embryo incubated somewhat more than a week, the growth of the lower jaw begins to outstrip that of the tongue very rapidly. The longitudinal ridges along the tongue become wider posteriorly and more prominent. The flat area behind them is more clearly delimited.

Subsequently, the rapid growth of the lower jaw draws out the frenulum linguae in length. The flat posterior portion of the tuberculum impar becomes delimited behind by a crescentic furrow with concavity directed anteriorly. Soon the posterior limiting furrow becomes continuous with the anterolateral furrow, so that the body of the tongue is clearly circumscribed. The median lingual groove now extends itself back almost to the posterior limiting furrow. The rudimentary epiglottis gradually becomes less distinct and returns to the status of a median ridge. It lies in the cranial part of a region that arches around the anterior end and sides of a bulging, pear-shaped area containing the glottis. The entire region surrounding the glottis and arytenoid ridges anterolaterally embraces the territory of the second and third visceral arches and comprises the base of the tongue (Fig. 179-F).

As the tongue becomes longer and wider, it overlaps the anterolateral limiting furrow (cf. Fig. 175-E). The frenulum linguae is brought beneath the tip of the tongue. Along the posterior limiting furrow, as well as along the lateral edges of the tongue, there appear protuberances which are the primordia of horny papillae. Similar structures develop along two narrow ridges that form close to the median groove and about midway between its



ends (Fig. 179-G). Papillae also form on the base of the tongue and around the glottis.

According to Kallius (1905), the development of the tongue in the sparrow (*Passer domesticus*) differs in a few respects from its development in the duck (*Anas platyrhynchos*). In the sparrow, the lateral lingual ridges fuse with each other across the anterior midline. The tuberculum impar is prolonged posteriorly at the sides rather than in the midline, hence the apex of the posterior limiting furrow is directed anteriorly instead of posteriorly; also, the furrow is not curved but forms an acute angle. The median groove does not appear until after the posterior limiting furrow has formed. The horny protuberances are distributed somewhat differently, and the sparrow hatches soon after the papillae begin to develop. The chicken's tongue resembles the sparrow's (*Passer domesticus*) more than the duck's (*Anas platyrhynchos*). In the hummingbird (*Trochilidae* sp.) and the honey eater (*Myzomela sclateri*), also, the tongue resembles the sparrow's until close to the end of incubation and acquires its distinctive structure during the nestling stage (Scharnke, 1931).

### *The Lingual and Other Oral Glands*

The first indication of the development of glands in the tongue is seen about the time that the anterior grooves begin to diverge from each other posteriorly rather than anteriorly. In the beginning, the glandular primordia are solid buds of epithelium that grow down toward the underlying mesoderm and rapidly increase in size. After the anlagen of horny papillae have begun to appear along the posterior limiting furrow and the edges of the tongue, there are, according to Kallius (1905), three sets of glands visible in the tongue of the duck (*Anas platyrhynchos*) embryo: the anterior lingual glands, between the longitudinal ridges and the edges of the tongue; the posterior lingual glands, on the base of the tongue, just behind the posterior limiting furrow; and the linguolaryngeal glands, on the base of the tongue and diverging posteriorly around the glottis. On the floor of the mouth under the tip of the tongue, there is a row of sublingual glands on either side of the midline. Numerous glands are also present on the wall of the pharynx at this time, those on the dorsal wall being especially large. In the sparrow (*Passer domesticus*) of the same developmental stage, the lingual glands are much more extensive. The sublingual glands are present anteriorly and extend back along the edges of the tongue and the floor of the mouth to join the glands on the pharyngeal wall. From a point about midway between the tip of the tongue and the posterior limiting furrow, the lateral lingual glands extend posterolaterally into the posterior angles of the tongue. Between these glands and extending as far caudad as the glottis, the medial lingual glands are disposed in a wide band, and behind these, on either side of the glottis, are numerous laryngeal glands. Reichel



(1883) stated that the first lingual glands appear in the chick embryo on the eighth day on the base of the tongue and in the angle formed between the tongue and the floor of the mouth.

### *The Musculature of the Tongue*

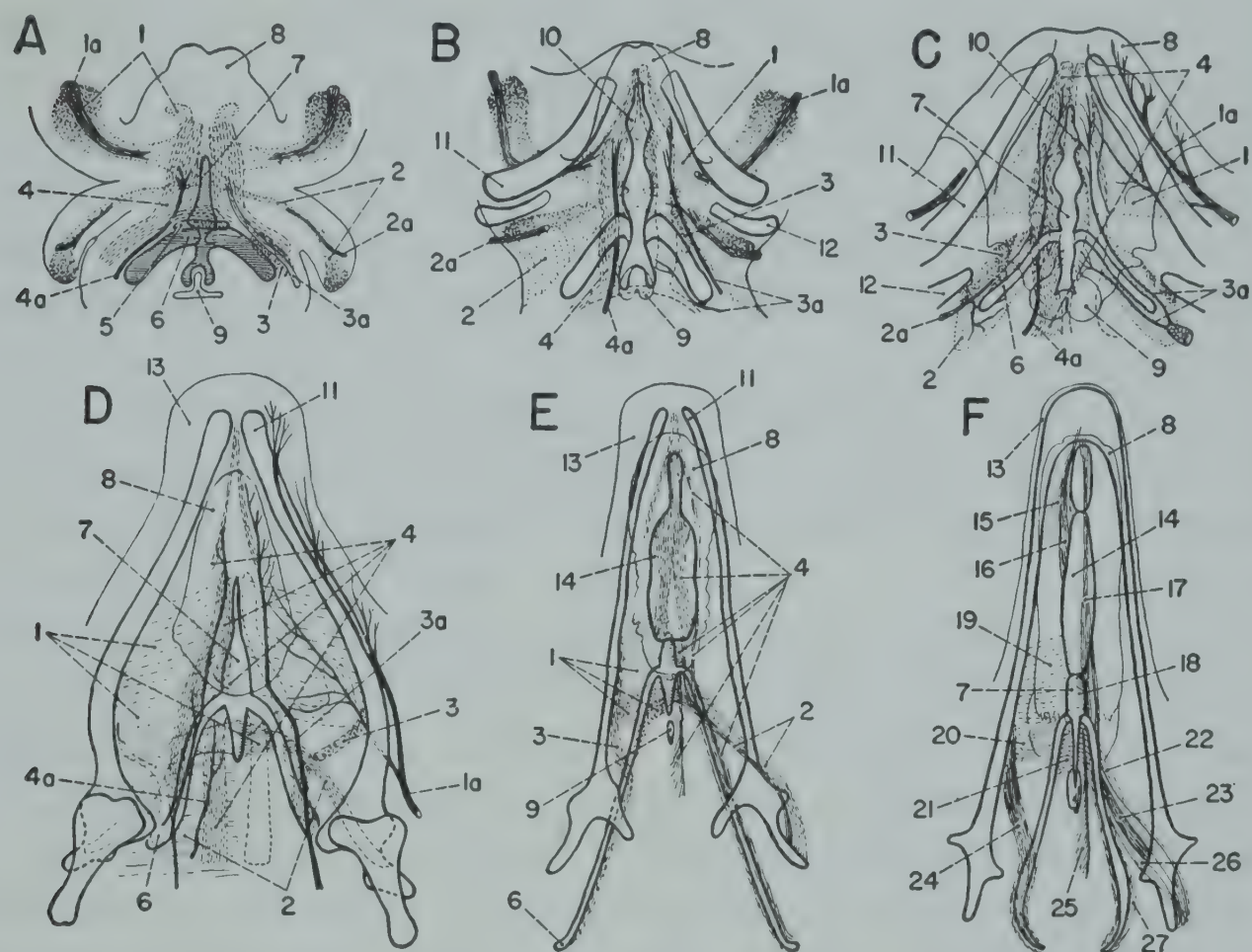
Four sets of muscles of different origin serve to move the tongue and to attach it to the mandibles and to the hyobranchial skeleton which consists of the bilateral cornua articulating in the midline with the copula. The copula, a longitudinal element formed by the fusion of the basihyal and the basibranchial (see Chapter 12), supports the base of the tongue. Extending forward from the copula is the paraglossum or entoglossum, another median element of cartilage or bone (depending upon the species), which supports the body of the tongue.

The muscles lying within the tongue itself pursue a generally longitudinal course close to the midline and are attached to the various elements of the hyobranchial skeleton. They are probably derived from material of the second to fourth or fifth pairs of somites which invades the floor of the pharynx along with, or shortly before, the hypoglossal (twelfth cranial) nerves. The remaining muscles associated with the tongue originate from the mesoderm of the ventral part of the first, second, and third visceral arches and are innervated by the trigeminal (fifth cranial), facial (seventh cranial), and glossopharyngeal (ninth cranial) nerves, respectively. The muscles arising from the first visceral arch run transversely across the floor of the mouth, and those arising from the second and third arches course obliquely between the posterior ends of the mandibles and the hyobranchial cornua.

Various stages in the development of the lingual musculature of the duck (*Anas platyrhynchos*) are represented more or less diagrammatically in Fig. 180, which is based on the observations of Kallius (1905). The first visible primordia of the hypoglossus musculature consist of bilateral condensations of cells, transverse to the long axis, that appear behind the median thyroid anlage shortly after the latter has migrated to the level of the second visceral pouch. After approaching very close to the midline, these muscle blastemas turn anteriorly and grow forward into the lingual region. When the cartilaginous precursors of the hyobranchial skeleton appear (see Chapter 12), the transverse muscle bundles lie beneath and parallel to the cornual portion (that is, the cartilages of the third visceral arches), and the two longitudinal muscle bundles are below and parallel to the copular portion (Fig. 180-A). Soon the longitudinal bundles grow far beyond the copular portion into the tongue primordium. They are also prolonged backward, posterior to the level of the cornua (Fig. 180-B and C), eventually to give rise to the tracheothyroid muscles. These muscles, which extend caudad along the trachea, are attached to the copula at the level of the



articulation between the latter and the cornua. Anterior to this articulation, the medial portions of the longitudinal muscle bundles become attached to the ventral surface of the copula (Fig. 180-B and C); later, from these attachments, fibers proceed to the medial ends of the cornua to form the posterior hyoglossal muscles (Fig. 180-F). At a still more anterior level,



**Fig. 180.** Stages in the development of the lingual muscles in the embryo of the duck (*Anas platyrhynchos*), represented diagrammatically as if projected upon a flat plane. (Redrawn with modifications after Kallius, 1905.)

A, from a 10.25-mm. embryo; B, from a 12.5-mm. embryo; C, from a 12.25-mm. embryo; D, from a 21.0-mm. embryo; E, from an embryo with a head length of 32.0 mm.; F, from an adult.

1, trigeminus musculature; 1a, trigeminal nerve; 2, facialis musculature; 2a, facial nerve; 3, glossopharyngeus musculature; 3a, glossopharyngeal nerve; 4, hypoglossus musculature; 4a, hypoglossal nerve; 5, thyroid primordium; 6, hyobranchial cornu; 7, copula of hyobranchial skeleton; 8, tongue; 9, laryngeal orifice; 10, lingual process of copula; 11, cartilage of first visceral arch; 12, cartilage of second visceral arch; 13, lower jaw; 14, paraglossum; 15, geniohyoid muscle; 16, genioglossus muscle; 17, anterior hyoglossus muscle; 18, posterior hyoglossus muscle; 19, mylohyoid muscle; 20, transverse hyomandibular muscle; 21, interceratoid muscle; 22, lateral hyomandibular muscle; 23, medial hyomandibular muscle; 24, ceratomandibular muscle; 25, tracheothyrohyoid muscle; 26, constrictor colli muscle; 27, ceratohyoid muscle.

the longitudinal bundles form medial attachments to the lingual process (cf. Fig. 180-B, C, and D) and gradually differentiate as the anterior hyoglossal muscles, which are attached to the ventral surface of the paraglossum and run forward to the extreme tip of the tongue (cf. Fig. 180-F). The lateral portions of the longitudinal bundles grow caudolaterad along the



hyoid cornua (Fig. 180-D) and eventually become the ceratohyoid muscles. These muscles extend from the caudolateral edges of the paraglossum to the caudal ends of the hyoid cornua (cf. Fig. 180-E and F). The most anterior part of the longitudinal bundles grows forward and downward below the tip of the tongue, to differentiate as the genioglossal muscles (cf. Fig. 180-D and F). Each of these muscles is attached anteriorly near the apex of the lower jaw and posteriorly to the edge of the paraglossum. Kallius (1905) distinguished a lateral portion of this muscle and termed it the geniohyoid muscle.

The muscle blastemas in the first three visceral arches appear at approximately the same time as the primordia of the hypoglossum musculature, but ventral to the latter. The paired cell condensations in the first and second arches are transverse to the long axis of the embryo. The members of each pair grow toward the midline, where they meet and fuse with each other. The median portions of the trigeminus and facialis musculatures then join together. The cell condensation in the third arch soon gives off a mass destined for the pharynx, and only a small muscle blastema remains near the lingual region. This small mass runs obliquely forward and medially and is associated with the lingual ramus of the glossopharyngeal nerve (cf. Fig. 180-A).

The muscle anlage in the first arch (that is, the primitive trigeminus musculature) grows forward in the midline with the mandibular process and is soon divided into a dorsal and a ventral portion by Meckel's cartilage, the primordial mandible (Fig. 180-B). The dorsal portion gives rise to the masseter muscle. The ventral portion is now situated in the anterior part of the floor of the mouth, extending close to the apex of the lower jaw (Fig. 180-C). By the time the anterior and lateral limiting furrows of the tongue become continuous, fibers have developed in this ventral part of the trigeminus musculature. The fibers run transversely from the mandible to a tendon in the midline and comprise the primitive mylohyoid muscle. The mandible now grows forward more rapidly than the muscle, which begins to assume a continually more posterior position (cf. Fig. 180-D). Definitively, the trigeminus musculature extends no farther forward than the midpoint of the mandible; its posterior limit, originally well anterior to the articulation of the basihyal and the hyoid cornua, eventually lies near the caudal end of the basibranchial (cf. Fig. 180-F). Meantime, the posterior part of the mylohyoid muscle splits horizontally to give off the transverse hyomandibular muscle dorsally (Fig. 180-E and F). A second horizontal fissure, dorsal to the first and more medial, produces the interceratoid muscle, which connects the medial aspect of each hyoid cornu with the raphe between the two transverse hyomandibular muscles (cf. Fig. 180-E and F). The latter muscles and the interceratoid muscles are connected posteriorly in the midline to the caudal end of the urohyal.



The muscle primordium in the second arch (the facialis musculature) is pulled forward medially and changed in direction from transverse to oblique as a result of the rapid regional growth in the midline. This muscle primordium originally consists of a long, narrow cell condensation, round in cross section, abutting laterally upon the medial side of the rudimentary cartilaginous bar of the second visceral arch (Fig. 180-B). Soon, however, the facialis musculature on each side becomes relatively more posterior in position and lies at the level of and parallel to the hyoid cornu. Simultaneously, the cellular strand broadens caudad to form a thin muscular sheet that extends down the neck to become the constrictor colli muscle (Fig. 180-C). The lateral portion of the cellular strand acquires an attachment to the caudal end of the mandible (Fig. 180-D). Fibers then proceed from this attachment along the lateral aspect of the hyoid cornu to its anterior end; these fibers make up the lateral hyomandibular muscle, which lies dorsal to the ceratohyoid muscle. The medial hyomandibular muscle then differentiates along the anterior edge of the primitive facialis musculature. Its fibers originate with those of the lateral hyomandibular muscle on the lateral side of the mandible and course ventral to the ceratohyoid muscle to meet fibers coming from the opposite side beneath the basibranchial (cf. Fig. 180-E and F).

At first, the small muscle blastema innervated by the glossopharyngeus lies close to the lateral end of the hyoid cornu (cf. Fig. 180-A). It then moves forward to a point medial to the cartilage of the second arch (cf. Fig. 180-B and C). As the hyoid apparatus becomes relatively more anterior in position, this muscle primordium becomes applied against the anterolateral side of the hyoid cornu. Eventually, the anterior end of the glossopharyngeus musculature swings laterad and becomes attached to the medial aspect of the mandible (cf. Fig. 180-D). The definitive muscle formed from this blastema is the ceratomandibular, proceeding from the midpoint of the mandible to the midpoint of the hyoid cornu and hence along the latter to its caudal end (cf. Fig. 180-F).

### *The Internal Structure of the Tongue*

Kallius (1905) described the internal organization of the duck (*Anas platyrhynchos*) embryo's tongue at a stage only a few days before hatching. Most of the hyoid apparatus, which forms the skeletal framework of the tongue, is still cartilaginous. The paraglossum is found throughout most of the body of the tongue. A horizontal septum of connective tissue extending from the upper surface of the paraglossum to each edge of the tongue divides the tongue into a dorsal and a ventral portion. The ventral portion is the thicker of the two, except posteriorly. It contains, in addition to the paraglossum, a row of large venous spaces situated directly under each of the horizontal septa. Anteriorly, the dorsal portion of the tongue is divided



longitudinally by a median septum extending dorsad from the paraglossum. In cross sections, the tissue on either side of the median septum is seen to be arranged in concentric layers. At the level where the tongue becomes attached to the floor of the mouth, the median septum disappears and the tissue layers are arranged about a single median center formed by the confluence of the two lateral centers. A sheath of connective tissue surrounds the layered complex and separates it from the more lateral regions. The dorsal and ventral portions of the tongue are both much thicker here than farther anteriorly. About at the midlevel of the body of the tongue, the concentric layers are replaced for a short distance by connective tissue, posterior to which they are again arranged around bilateral centers. Fatty tissue occupies the dorsal median part of the tongue at this level. In the vicinity of the posterior end of the paraglossum, the fatty tissue and the layered configurations both disappear, and the mylohyoid muscle lies between the tongue and the floor of the mouth.

### Possible Vestigial Teeth

The presence of teeth in the fossils of certain Jurassic and Cretaceous birds has led to a search for dental vestiges in modern birds, all of which are toothless. The first structures to be considered as rudimentary teeth were horny papillae found on the margins of the beak in advanced embryos of the Indian ring-neck parrot, *Psittacula krameri manillensis* (Geoffroy St. Hilaire, 1821), and in nestlings of various species of parakeets and cockatoos (Blanchard, 1860; Fraisse, 1880). Beneath the papillae are conical structures supplied with blood vessels and nerves and resting against the periosteum of the mandible and maxilla. Blanchard (1860) believed that these conical structures produce dentine, and stated that bone eventually grows over them. Fraisse (1880), however, showed that they are composed of connective tissue and keratin; he then proposed that the teeth of the first birds may have consisted of calcified keratin. The only other possible dental rudiment in birds is the "dental ridge," so-called because of its resemblance to the first primordium of teeth in mammals. This structure has been seen in embryos of a number of avian species, including the ostrich, *Struthio camelus* (Röse, 1892); the common tern, *Sterna hirundo* (Röse, 1892; Carlsson, 1896; Tjeenk Willink, 1899); the Sandwich tern, *Sterna sandvicensis*; the European gallinule, *Gallinula chloropus*; the thick-knee or stone curlew, *Burhinus oedicephalus*; the curlew, *Numenius* sp. (Tjeenk Willink, 1899); and the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901; Keibel, 1901). In the avian embryo, the "dental ridge" is an epithelial thickening near the edge of the beak, projecting toward the mesoderm. It gradually flattens out and disappears.

Ihde (1912) objected to interpreting either the papillae or the epithelial ridge as dental vestiges. In his estimation, the papillae bear no structural



or genetic resemblance to teeth, and Fraisse's theory is improbable in the light of palaeontological and phylogenetic evidence. The "dental ridge" appears too late in development and is too wide to be equivalent to the mammalian structure; furthermore, it is highest anteriorly, in the region where teeth had already disappeared in the remote ancestors of birds. The presence of several additional epithelial ridges medial and parallel to the "dental ridge" (*Röse, 1892; Carlsson, 1896; Tjeenk Willink, 1899*) led Ihde (1912) to the conclusion that these structures are all equivalent formations related to the process of keratinization of the beak.

## THE ESOPHAGUS AND THE CROP

The esophagus is the portion of the digestive tube leading from the oral cavity and pharynx to the proventriculus, or stomach. Anteriorly, the esophagus is dorsal to the trachea, but, in proceeding downward, it curves somewhat to the right of the latter. Massive longitudinal ridges, or rugae, project into the lumen of the esophagus from the inner surface of its wall. The passage of food through the esophagus is facilitated by its great distensibility, by the contractions of muscles in its walls, and by the secretions of mucous glands. Somewhat below its midlevel, the esophagus expands locally to form the crop, in which food is temporarily stored and softened.

### Gross Development of the Esophagus

The esophagus elongates rapidly as the neck grows, but does not quite keep pace with the latter; hence the course of the esophagus (and trachea) is straighter than that of the cervical vertebrae, especially in passerine birds (*Niethammer, 1933*). During the course of development, the esophagus gradually bends to the right, then, at the bifurcation of the trachea, it turns to the left to enter the stomach. This somewhat spiral course is apparent by the chick's twelfth day of incubation (*Goette, 1867*).

Incubation Age (days)	Diameter of Esophagus (mm.)	Thickness of Esophageal Wall (mm.)
7	0.24	
9	0.35	0.05
12	0.50	0.09
16	0.58	0.16
20	1.60	0.33

As the esophagus grows in length, it also increases in diameter. The tabulated measurements of the chick embryo's esophagus (*Livan, 1951*) indicate the changes in its diameter and in the thickness of its wall during the second and third weeks of incubation. The lumen of the esophagus is occluded or extremely small in early stages of development, then briefly



circular, and eventually almost filled by the projecting rugae. The changes in the size and shape of the lumen, however, are reflections of the histological development of the esophageal wall.

### Histological and Functional Development of the Esophagus

The only portions of the esophagus that are derived from the endoderm of the fore-gut are its lining of stratified squamous epithelium and its mucous glands. The more peripheral portion, consisting of connective tissue and muscle, differentiates from splanchnic mesoderm. Immediately outside of the epithelium is a layer of connective tissue, the tunica propria, which forms the bulk of the rugae and within which the mucous glands are embedded. Superficial to the tunica propria is a longitudinal layer of smooth muscle, the muscularis mucosae, which usually extends into the rugae. In some birds, including gallinaceous species, the muscularis mucosae is surrounded by another layer of connective tissue, the submucosa. External to either the muscularis mucosae or the submucosa is a ring of smooth muscle with fibers disposed circumferentially. When a submucosa is present, there is usually a thin layer of longitudinal muscle peripheral to the circular layer. The outermost layer, or adventitia, consists of connective tissue joined with the more medial connective tissue by fibers that pass through the external muscularis. Two ganglionated autonomic plexuses, Meissner's plexus and Auerbach's plexus, lie in the submucosa and the external muscularis, respectively; they are connected with the vagus nerve. A serosa is present only around the thoracic portion of the esophagus.

#### *The Epithelium*

In birds, as in other vertebrates except mammals (*Forssner, 1907; Pensa, 1910*), the upper part of the esophagus becomes occluded soon after its separation from the trachea. Obliteration of the esophageal lumen has been observed in embryos of the chicken (*Meuron, 1886a; Keibel and Abraham, 1900; Forssner, 1907; Pensa, 1910; Schumacher, 1926; Trotti, 1932*); the duck, *Anas platyrhynchos* (*Trotti, 1933c*); the zebra parakeet, *Melopsittacus undulatus* (*Abraham, 1901; Joos, 1941*); the European lapwing, *Vanellus vanellus* (*Grosser and Tandler, 1909*); the least tern, *Sterna albirostris*; and the European goldfinch, *Carduelis carduelis* (*Pensa, 1910*).

The occlusion of the esophagus proceeds from anterior to posterior and is caused by the proliferation of the epithelial lining. In the 3-day chick embryo, the esophagus is round in cross section, and the diameter of its lumen exceeds the thickness of its endodermal walls (*Meuron, 1886a*), in which there is more than one layer of nuclei (*Forssner, 1907*). During the fourth day, the nuclei start to divide actively, beginning at the cranial end of the esophagus, where the lumen immediately becomes narrower (*Forssner, 1907*). In the chick (*Schumacher, 1926*) or zebra parakeet



(*Melopsittacus undulatus*) embryo (Joos, 1941), the esophageal epithelium is thick and contains two or three layers of nuclei at the end of the fourth day. Between the middle and end of the chick's fifth day, the epithelium increases in thickness from 40 microns to 50 or 55 microns (Meuron, 1886a; Schumacher, 1926). During this same period, the lumen of the anterior esophagus decreases in diameter from 18 microns to 7.5 microns; 12 hours later, it has disappeared completely for a distance of 115 microns posterior to the junction of esophagus and trachea (Meuron, 1886a). A few investigators have found, however, that occlusion of the anterior part of the chick's esophagus may occur somewhat earlier, at the end of the fifth day (Keibel and Abraham, 1900; Forssner, 1907; Pensa, 1910; Schumacher, 1926), or even at the end of the fourth day (Trotti, 1932). Occlusion has been observed on the fifth day in the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901; Joos, 1941), on the seventh day in the duck, *Anas platyrhynchos* (Trotti, 1933c), and at the 51-somite stage in the European lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909).

According to Forssner (1907) and Pensa (1910), the proliferation of epithelium progresses caudad during the chick's sixth day until the esophageal lumen is obliterated down to the level of the cranial border of the eighth vertebra or halfway to the bifurcation of the trachea. Below the crop, which is a slight dilatation at this time, the lumen of the digestive tube does not disappear, but becomes extremely small (Trotti, 1932). By the middle or end of the sixth day, the posterior part of the dorsoventrally flattened pharynx has been added to the interior end of the esophagus, and this portion also is occluded (Meuron, 1886a; Forssner, 1907; Trotti, 1932).

The cavity of the esophagus begins to re-establish itself from posterior to anterior (Meuron, 1886a) during the chick's seventh day (Forssner, 1907; Pensa, 1910; Trotti, 1932). The lumen of the lower or thoracic esophagus starts to widen, and lacunae appear in the obliterated upper portion. Anteriorly, where the esophageal epithelium forms a flat plate, the lacunae develop laterally; more posteriorly, they tend to resolve into two lateral cavities separated by a septum containing smaller lacunae; still farther caudad, the two cavities join to form a single lumen which continues into the crop (Meuron, 1886a; Forssner, 1907; Pensa, 1910; Trotti, 1932). The confluence of the small cavities extends the undivided lumen cranial until finally only the flat epithelial plate contains multiple cavities separated by epithelial trabeculae. When the latter disappear, the esophagus is once more open continually from end to end. According to Forssner (1907) and Pensa (1910), the chick's esophagus becomes luminate at the end of the eighth day or the middle of the ninth, but Trotti (1932) stated that the last remnants of the trabeculae persist until the end of the eleventh day. The esophagus is reopened by the ninth day in the zebra parakeet,



*Melopsittacus undulatus* (Joos, 1941), and by the thirteenth day in the goose, *Anser anser* (Fig. 181).

As the lumen of the esophagus begins to reappear, the cells rearrange themselves into a single layer, and there is a rather abrupt decrease in the thickness of the epithelium; thus, in the 7-day chick embryo, the epithelium is only 25 microns thick (Schumacher, 1926). About at the midpoint of the incubation period, the number of cell layers once more begins to increase. In the chicken and the turkey (*Meleagris gallopavo*), four to eight layers are present 2 or 3 days before hatching; in the guinea fowl (*Numida*

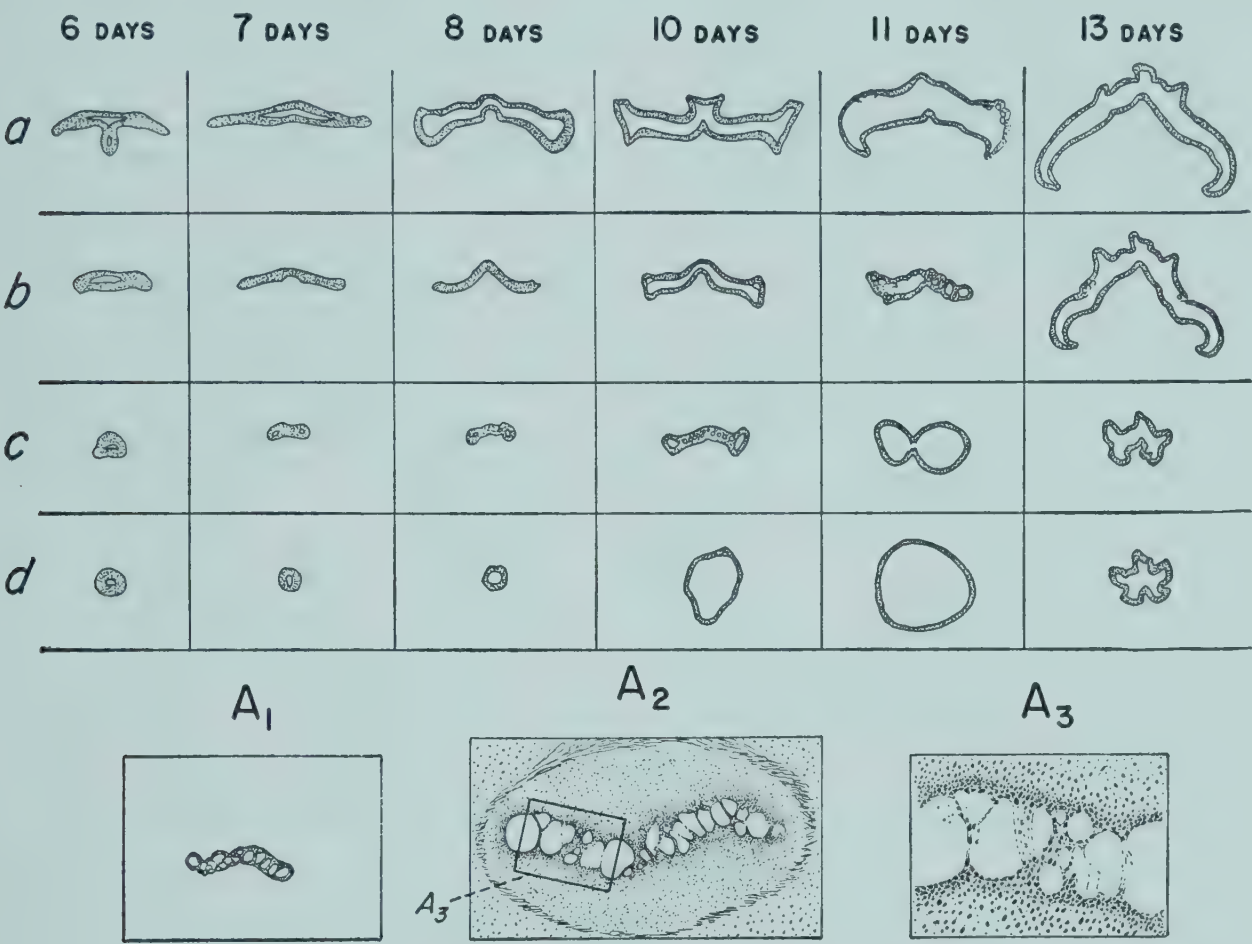


Fig. 181. Cross sections of successive levels of the esophagus of the developing goose (*Anser anser*) embryo. (Redrawn and rearranged after Trotti, 1933c.)

a, b, c, and d, cross sections at successively more caudal levels of the esophagus, from embryos incubated from 6 to 13 days.

A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>, cross sections at successively higher magnifications, of the reopening esophagus in the 11-day embryo, showing lacunae separated by epithelial ridges.

*meleagris*), pigeon (*Columba livia*), and zebra parakeet (*Melopsittacus undulatus*), only two to five layers are seen at a comparable incubation stage (Joos, 1941; Ivey and Edgar, 1952). The epithelium is somewhat thicker in the lower than in the upper esophagus (Ivey and Edgar, 1952). Schumacher (1926) found more cell layers between the rugae than upon their apices. As the epithelium again becomes stratified, the cells decrease in height and become cuboidal, hence the maximum thickness of the epithelium is no greater on the nineteenth day, in the chick, than on the sixth (Schumacher, 1926). A flattening of the superficial epithelial cells is apparent about midway through the incubation period in the tufted pen-



guin, *Eudytes chrysolophus* (Bartram, 1901) and the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941), but not until the eighteenth day in the chick (Schumacher, 1926).

During the last 24 hours before hatching, the number of epithelial cell layers very rapidly increases to ten to twenty in the turkey (*Meleagris gallopavo*) and the chick and to six to eight in the pigeon (*Columba livia*). A further increase to twenty-five layers (or more) occurs shortly after hatching in the chicken (Schumacher, 1926), the guinea fowl (*Numida meleagris*), and the turkey, *Meleagris gallopavo* (Ivey and Edgar, 1952).

It has been reported that the superficial epithelial cells of the esophagus exhibit cilia when the epithelium is two to four layers thick, that is, on the thirteenth, sixteenth, and twenty-third day of incubation in the chicken, turkey (*Meleagris gallopavo*), and guinea fowl (*Numida meleagris*), respectively. Ciliation is not extensive except in the region near the proventriculus. It is most marked in the turkey, in whose lower esophagus a maximum of 90 to 100 per cent of the cells may be ciliated, as compared with 50 per cent in the chick and 25 per cent in the guinea fowl. The cilia disappear on the last day of incubation or the first day after hatching and persist longest in the upper esophagus (Edgar, 1947; Ivey and Edgar, 1952).

**The Mucous Glands.** The mucous glands of the esophagus take a variety of forms in different species, but in all birds they develop from buds of epithelium that project into the tunica propria. In the chicken, the turkey, *Meleagris gallopavo*, the guinea fowl, *Numida meleagris* (Ivey and Edgar, 1952), and the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941), these buds appear when development is 60 to 65 per cent completed; but there is no trace of them at a comparable stage in the tufted penguin, *Eudytes chrysolophus* (Bartram, 1901), and they do not form in the pigeon (*Columba livia*) until 80 per cent of the incubation period has passed (Ivey and Edgar, 1952). Gland formation begins at the caudal end of the esophagus and progresses cranial (Schreiner, 1900; Joos, 1941); in some species (including the zebra parakeet, *Melopsittacus undulatus*), the glands are confined to the lower esophagus.

The epithelial buds, most of which lie in the depths between the rugae (Schreiner, 1900; Schumacher, 1926), are at first solid (Fig. 182-A; cf. Fig. 183-D and E). In the 18-day chick embryo, the buds exhibit secondary projections, and lumina are beginning to develop within them. The gland primordia become hollow through the formation and confluence of intercellular vacuoles (Fig. 182-B<sub>1</sub>). Cavitation proceeds toward the lumen of the esophagus. The accumulation of extracellular fluid within the developing glands produces vesicles beneath the esophageal epithelium (cf. Fig. 182-B<sub>1</sub>). In the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941), and the sparrow hawk, *Accipiter nisus* (Niethammer, 1933), this fluid stains with mucicarmine, but Schumacher (1926) could not demonstrate its



mucinous nature in the chick embryo. The pressure of the accumulated fluid causes the esophageal epithelium to stretch, grow thinner, and finally to rupture (Fig. 182-B<sub>2</sub> and C). In the 19-day chick embryo, the glands are formed of cylindrical cells containing granules of premucin or mucinogen (Schumacher, 1926; Ivey and Edgar, 1952). Gland formation is still in progress at the time of hatching.

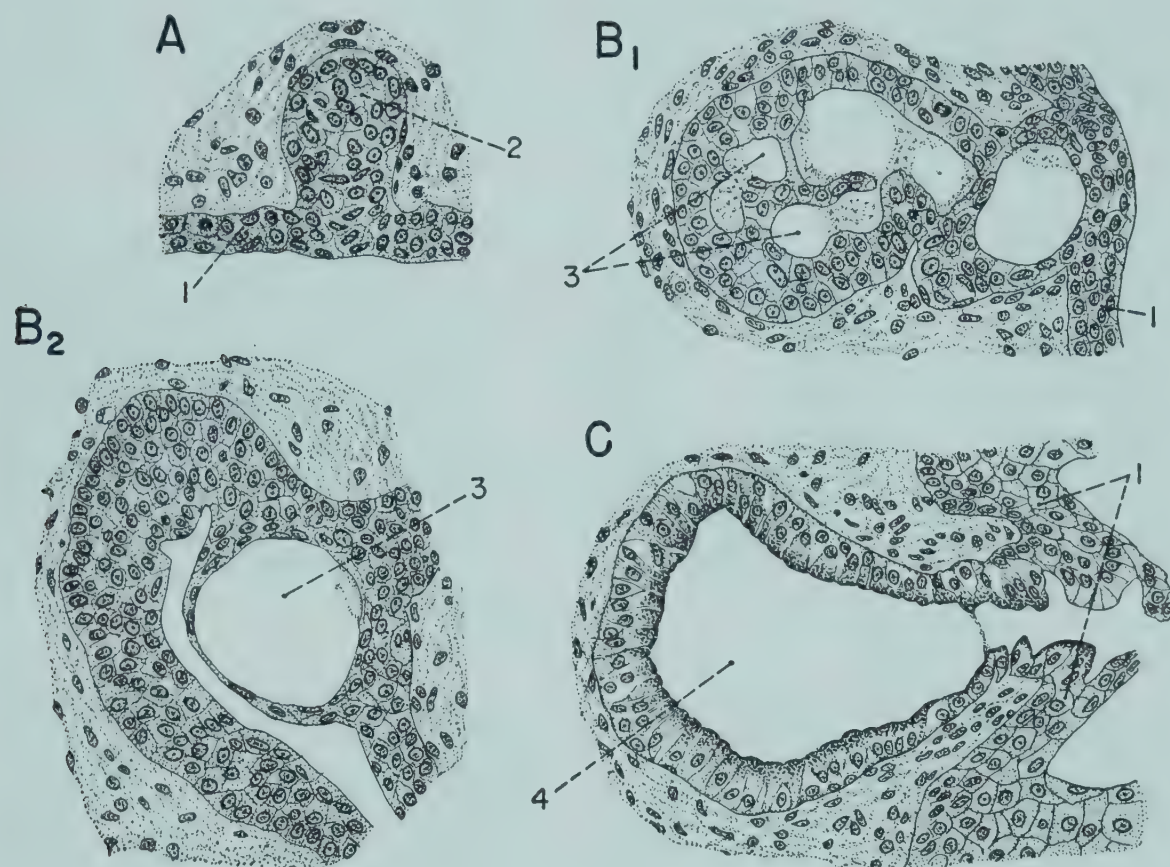


Fig. 182. Mucous gland development in the esophagus of the chick embryo. (Redrawn with modifications after Schumacher, 1926.)

A, mucous gland from a 16-day embryo; B<sub>1</sub>, B<sub>2</sub>, the same, from an 18-day embryo; C, the same, from a 19-day embryo. All  $\times 250$ .

1, esophageal epithelium; 2, solid epithelial bud; 3, vacuoles; 4, lumen of gland.

### The Muscle and Connective Tissue

Differentiation of the mesodermal portion of the esophagus may begin when incubation is roughly one fifth to one third completed; it has been observed on the fifth incubation day in the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941) and on the fourth to seventh day in the chick (Forssner, 1907; Gozzi, 1940; Livan, 1951). The cells immediately external to the epithelium become densely packed; these cells represent the tunica propria (Gozzi, 1940; Ivey and Edgar, 1952). At some distance from the epithelium, external to a zone of loose connective tissue, the elements of the future circular muscle layer elongate slightly and orient themselves circumferentially (Gozzi, 1940; Ivey and Edgar, 1952).

As the epithelial occlusion of the esophagus starts to resolve, the intermediate loose connective tissue begins to grow thicker and thus to form the first indication of rugae. A cross section of the 7-day chick embryo's



esophagus shows that the lumen has the shape of a five-pointed star (Trotti, 1932). This stage is seen in the 10-day turkey (*Meleagris gallopavo*) embryo (Fig. 183-A).

The outer longitudinal muscle layer (if it is to form) becomes recognizable soon after the circular layer (Fig. 183-B). It is visible in the 9-day chick (Livan, 1951) and the 13-day turkey (*Meleagris gallopavo*) embryo (Ivey and Edgar, 1952).

By the approximate midpoint of incubation, secondary ridges have begun to form between the primary rugae (cf. Fig. 183-B), and there is a com-

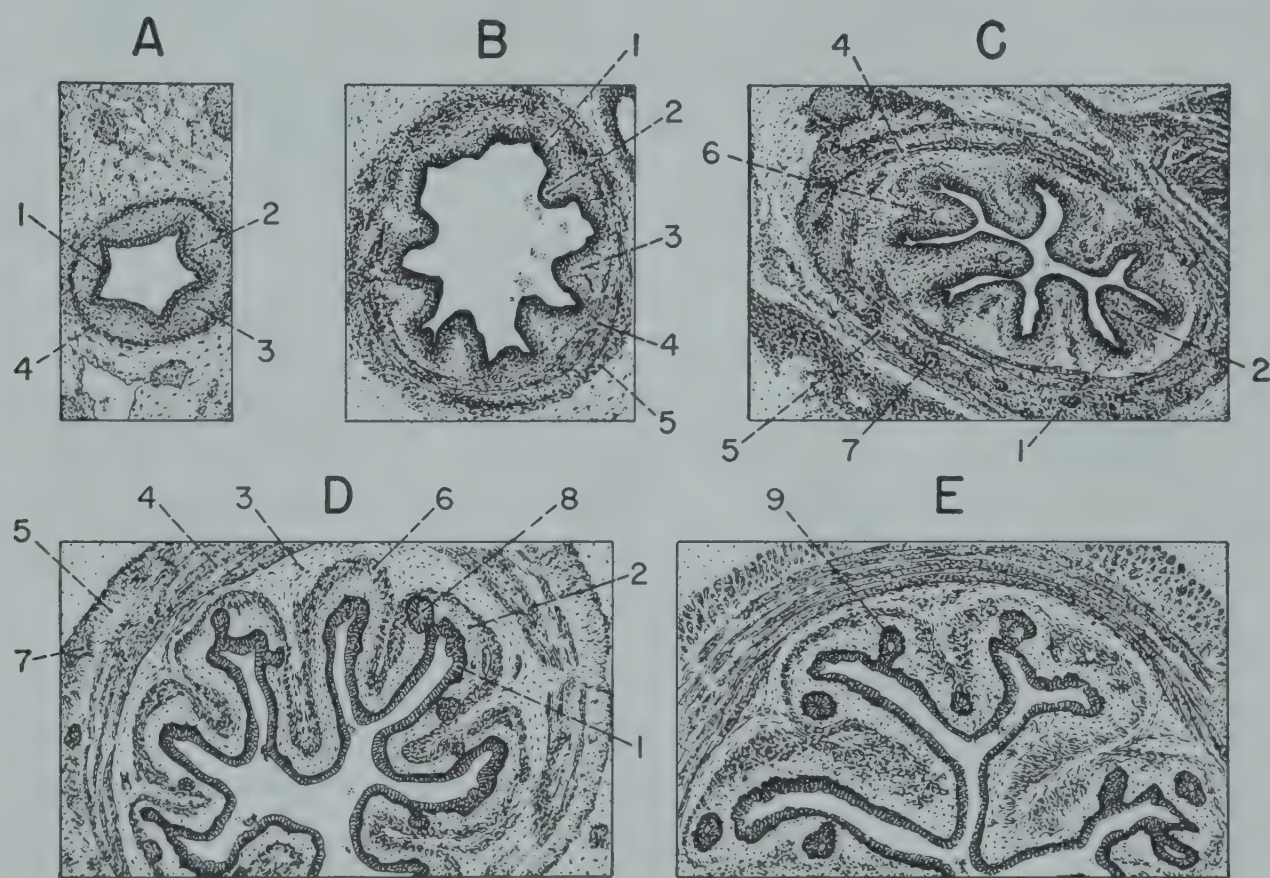


Fig. 183. Stages in the development of the esophagus in the turkey (*Meleagris gallopavo*) embryo, shown in cross section. (Redrawn with modifications after Ivey and Edgar, 1952.)

A, at 10 days; B, at 13 days; C, at 16 days; D, at 20 days; E, at 23 days. All  $\times 40$ .

1, epithelium; 2, tunica propria; 3, submucosa; 4, circular muscle layer; 5, longitudinal muscle layer; 6, muscularis mucosae; 7, ganglion of Auerbach's plexus; 8, epithelial bud (primordium of mucous gland); 9, mucous gland with lumen.

plete system of compact circular muscle fibers (Livan, 1951). The muscularis mucosae now becomes identifiable. In the turkey, *Meleagris gallopavo*, the chicken, the guinea fowl, *Numida meleagris* (Ivey and Edgar, 1952), and the tufted penguin, *Eudyptes chrysolophus* (Bartram, 1901), this muscle layer usually projects into the rugae from the start. In these birds, its differentiation makes it possible to distinguish the submucosa from the tunica propria. As the tunica propria grows thicker, the muscularis mucosae gradually assumes a position midway between the epithelium and the circular muscle layer (Fig. 183-D and E). In the zebra parakeet (*Melopsittacus undulatus*), which definitely lacks a submucosa, the muscu-



laris mucosae is plainly visible on the ninth day of incubation as a band of cells in the midst of the connective tissue, parallel to the circular muscle layer; it gradually moves outward until it lies directly internal to the circular layer, and not until then does it begin to extend medially into the rugae (Joos, 1941).

During the second half of incubation, the increasing size of the rugae transforms the lumen of the esophagus, as seen in cross section (cf. Fig. 183), into several narrow, radially arranged fissures. The tunica propria and the muscularis mucosae become vascularized during this period (Ivey and Edgar, 1952). In the chick, the thickness of the muscle layers eventually increases to about two thirds of the total thickness of the wall (Livan, 1951).

The development of contractile activity has been studied by means of cultures of the explanted esophagus or entire fore-gut complex (esophagus, trachea, and stomach). The 4- to 5-day chick embryo's esophagus may contract spontaneously after 6 to 36 hours of cultivation *in vitro* (Gozzi, 1940; Staudacher-Dalle Aste, 1941; Keuning, 1948). The onset of contractility coincides with the initial differentiation of myoblasts (Staudacher-Dalle Aste, 1941). Different observers have said that the first contractions appear at the lower end of the esophagus (Staudacher-Dalle Aste, 1941), at the cranial end (Keuning, 1948), and at the point where the esophagus crosses the left bronchus (Jong and Haan, 1943). Although weak and irregular in the beginning, they quickly become rhythmical and exhibit a peristaltic character (Staudacher-Dalle Aste, 1941); in perfused cultures of the undivided fore-gut complex, the contractions develop within 24 hours to complete peristaltic waves lasting 20 to 25 seconds and coming in groups of three or four every 10 to 30 minutes (Jong and Haan, 1943). In cultures of the esophagus from older (6- to 10-day) embryos, the frequency of contraction is higher, the duration of the contractile phase is less, and the velocity of the peristaltic wave is greater (Staudacher-Dalle Aste, 1941).

The cultured esophagus gives a weak local response to mechanical stimulation. Electrical stimuli have no effect until after the onset of spontaneous contractility. Faradic stimulation for 2 minutes may then increase the frequency of contraction by 100 per cent. Doubling of the contraction rate may also be observed when the temperature is elevated from 39° C. to 41° to 43° C. (Staudacher-Dalle Aste, 1941). Weak solutions of drugs, such as adrenalin, pilocarpine, and tropine, do not exert their characteristic effects upon contractile activity in esophagus cultures taken from chick embryos less than 9 or 10 days old. Staudacher-Dalle Aste (1941) related this finding to the fact that not until the tenth day could he demonstrate vagus fibers deep in the esophageal wall and connected with the autonomic plexus.

In the living embryo, also, the muscular activity of the esophagus begins at an early date. Kuo (1932b) found that only a small proportion (8 per



cent) of chick embryos perform the motions of swallowing on the eighth day of incubation, and that about one third of them do so on the thirteenth day. It should be pointed out, however, that swallowing is not a function of the esophagus alone.

Whether the initial contractions in the esophageal wall are myogenic or neurogenic is uncertain. Staudacher-Dalle Aste (1941) found that differentiating ganglion cells, interconnected by delicate fibers, were present in the muscle tunic of the cultured esophagus at the time contractility developed, and he therefore concluded that the myoblasts were subject to some neural regulation. Keuning (1948), on the other hand, observed contractions when the neuroblasts were too primitive, in his opinion, to exert any specific action. The peristaltic contractions, however, are without doubt under nervous control.

**Innervation of the Esophagus.** In the 5-day chick embryo, the vagus nerve gives off bundles of fibers to various levels of the esophagus. These bundles divide and subdivide on the surface of the muscle tunic and end by penetrating part of the way into it. According to Staudacher-Dalle Aste (1941), the vagus gives off fibers more abundantly at the lower end of the esophagus, where the fibers form a fairly complicated plexus with the differentiating autonomic plexus. In the 8-day embryo, the plexus of Auerbach is established and has numerous connections with the vagus fibers. This plexus is quite extensive in the 10- to 11-day embryo and its fibers are of different calibers. The ganglion cells are in large part multipolar (Staudacher-Dalle Aste, 1941). The ganglia of Meissner's plexus appear at the 13-day stage (Ivey and Edgar, 1952).

### Crop Formation

The crop is essentially a local expansion of the esophagus, which it resembles in histological structure. It is situated to the right of the trachea and its size and shape vary in different birds. There are two fundamental types of crop: spindle-shaped and saclike (Gadow and Selenka, 1891). The spindle-shaped crop may be merely a symmetrical dilatation of the esophageal lumen, as in birds of prey (Fig. 184-A), or it may consist also of a thickening of the wall, as in passerine birds. The sharply demarcated saclike crop, typified by that of galliform birds (Fig. 184-B), may assume an asymmetrical, somewhat spiral shape, as in the hoatzin, *Opisthocomus hoazin* (Fig. 184-C), and in psittaciform birds; in pigeons (*Columba livia*), it is composed of two large bilateral pouches (Fig. 184-D); and in hummingbirds (*Trochilidae*), it is a dilatation of the left side of the esophagus, rather than of the ventral or right ventrolateral side (Niethammer, 1933). The characteristic form of the crop becomes apparent at a fairly early developmental stage (Swenander, 1899; Böker, 1927).

The first indication of crop formation is a widening of the lumen of the



esophagus. In the chick, the future site of the crop is detectable on the fourth day of incubation, when a short section of esophagus exhibits a clearly circular lumen at a level slightly above the bifurcation of the trachea, the lumen elsewhere being occluded or very small (Trotti, 1932). The first true dilatation of the esophagus is seen in the 6-day chick (Trotti, 1932) or zebra parakeet, *Melopsittacus undulatus* (Niethammer, 1933), embryo; in the latter, the lumen of the crop is markedly oval. By the end of the chick's seventh day of incubation, the crop evagination is large enough to be grossly visible as a bend to the right in the region of the seventh cervical vertebra.

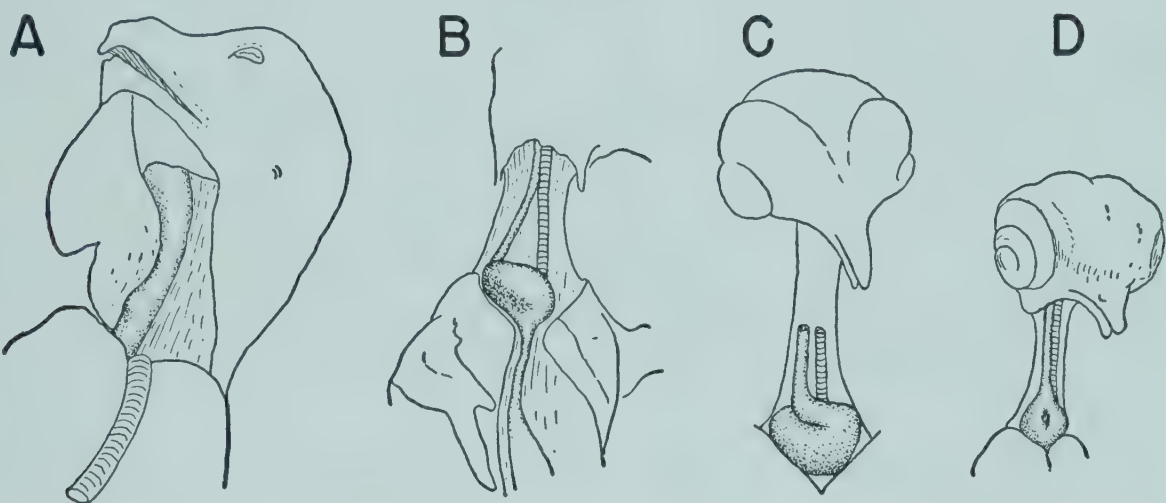


Fig. 184. Species differences in the position and form of the fully differentiated crop of four avian embryos at about the middle of their developmental periods. (A, B, and D, adapted from Swenander, 1899; C, from Böker, 1927.)

A, the slightly demarcated, symmetrical crop of the kestrel (*Falco tinnunculus*); B, the asymmetrical, well-developed crop of the chick (*Gallus gallus*); C, the large, convoluted crop of the hoatzin (*Opisthocomus hoazin*); D, the bilateral, symmetrical, well-developed crop of the pigeon (*Columba livia*). All  $\times 1.5$ .

The lumen in the evaginated region is 0.24 mm. wide, as compared with 0.16 mm. in the remainder of the esophagus. The table indicates the subsequent increase in the external diameter of the chick's crop (Mayberry, 1935). In the canary (*Serinus canaria*), the diameter of the crop does not increase much more rapidly than that of the esophagus, and the crop is only about 20 per cent wider than the esophagus at the time of hatching; in the pigeon (*Columba livia*), the bilateral pouches of crop begin to evaginate markedly on the eighth day of incubation, and the crop is nearly eight times as wide as the esophagus at the end of the developmental period (Niethammer, 1933).

Incubation Age	Diameter of Crop
(days)	(mm.)
8	0.7
9	1.6
10	3.0
16	5.0
21	8.0



On the chick's ninth day of incubation, the crop appears as an evagination of the right ventrolateral wall of the esophagus, which, at this time, is only one half as thick as the left or dorsomedial wall (*Livan, 1951*). On the tenth day, the evaginated wall is further reduced to one fourth the thickness of the nonevaginated wall (*Mayberry, 1935*). No glands develop in the evaginated wall (*Mayberry, 1935; Ivey and Edgar, 1952*), and its rugae, which are very few in number, do not appear until the thirteenth or fourteenth day (*Mayberry, 1935*). In other birds, such as the canary (*Serinus canaria*), the sparrow hawk (*Accipiter nisus*), and the zebra parakeet (*Melopsittacus undulatus*), the developing crop is as rugose as the esophagus (*Niethammer, 1933*).

The histological development of the crop wall parallels that of the esophagus in every important particular. In the chick, the muscular tunic of the evaginated portion becomes reduced in thickness until it is only one fourth as thick as the muscular tunic of the esophageal wall (*Livan, 1951*). *Niethammer (1933)* noted that the epithelium lining the pigeon (*Columba livia*) embryo's crop becomes thin dorsally and ventrally and thick in the bilateral pouches.

The crop has a more extensive nerve supply than the esophagus (*Ivey and Edgar, 1952*). In the 10- to 11-day chick embryo, many large nerve tracts run longitudinally. These are connected by smaller anastomotic tracts, so that a large-meshed plexus (of Auerbach) is formed (*Staudacher-Dalle Aste, 1941*).

## THE STOMACH

In birds, the stomach consists of two portions, the glandular stomach, or proventriculus, and the muscular stomach, or gizzard, which are distinct from each other both morphologically and physiologically. The proventriculus is a fusiform enlargement of the digestive tube situated at the lower end of the esophagus. Its walls are thick and contain numerous glands, which secrete digestive enzymes and mucin. The gizzard, immediately below the proventriculus, is a round, somewhat flattened organ with very thick, preponderantly muscular walls. The simple tubular glands of its lining are covered with a layer of secreted keratinoid material, which apparently serves as protection against the abrasive action of ingested pebbles and other hard, sharp objects. The gizzard secretes no digestive enzymes, and its function is merely the mechanical one of grinding; its muscular activity thus replaces mastication. The relative size of the gizzard is greatest in birds that feed upon plants and seeds, least in birds of prey. In some birds (for example, *Colymbiformes, Pelecaniformes*), a third or pyloric division of the stomach is recognizable (*Cattaneo, 1884*).



### Gross Development of the Stomach

The development of the stomach complex begins with the formation of an enlargement of the fore-gut immediately anterior to the liver diverticula. In the chick embryo, the stomach primordium apparently may differentiate at any time between the 48- and 72-hour stages (Goette, 1867; Keibel and Abraham, 1900; Maurer, 1906; Sjögren, 1941), that is, during the period when the twenty-seventh to thirty-sixth somites form. The initial appearance of the gastric anlage has been observed in the 30-somite zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901), and the 33-somite European lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909).

The first grossly visible changes in the stomach are a shift in its position toward the left and its division into an anterior and a posterior portion by the formation of a slight indentation in its wall. In the chick, deviation from the midline may occur at the 39-somite (72-hour) stage (Keibel and Abraham, 1900) or early in the fourth day (Maurer, 1906). The initial delimitation between proventriculus and gizzard appears soon afterward. It has been seen in the 51-somite lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909), and the 4.5- to 5-day zebra parakeet, *Melopsittacus undulatus* (Joos, 1941).

The leftward migration of the stomach complex becomes more pronounced after the chick's fifth day of incubation (Goette, 1867), chiefly because of pressure from the enlarging liver (Sjögren, 1941). The gizzard is affected more than the proventriculus. In the 6-day chick embryo, the right end of the greater curvature of the gizzard has evaginated to form a blind pouch directed caudad. As a result, the pylorus has moved cranial and lies to the right of the mouth of the proventriculus and at the same level (Sjögren, 1941). Figure 185-A gives an external view of the endodermal portion of the 6-day chick's stomach.

In the following days, the proventriculus becomes convex dorsally and flattened ventrally and is clearly separated from the gizzard by a constricted intermediate zone (Cattaneo, 1884). The caudal blind pouch of the gizzard bulges to the left as well as to the right, then elongates, and finally assumes a rounded form (Fig. 185-B, C, and D). After the chick's eleventh day, the gizzard gradually becomes lenticular in shape (Goette, 1867; Sjögren, 1941) as heavy muscles develop in its lateral walls. Eventually, it turns somewhat obliquely, so that its original ventral surface faces ventrolaterally to the left and the lateral muscles become practically dorsal and ventral.

The gizzard grows at a greatly accelerated rate after the thick lateral muscles begin to develop. Its weight is 0.025 to 0.035 gm. on the chick's ninth or tenth day (Schmalhausen, 1926; Dumm and Levy, 1949), 0.4 gm. on the sixteenth day (Latimer, 1928), and from 1.4 gm. to 2.0 or 2.5 gm. at



hatching (Latimer, 1925, 1928; Romanoff, 1933; Kuo and Shen, 1936; Fronda and Marcelo, 1938), when the proventriculus weighs only 0.4 gm. (Latimer, 1925; Fronda and Marcelo, 1938). Between the tenth and the fiftieth days, the proportion of fat-free solids (consisting chiefly of protein) in the gizzard rises abruptly from 8 per cent of wet weight to 14 per cent, a change which probably reflects the relative increase in the amount of muscle (Dumm and Levy, 1949). Incubation at a temperature of 36° C. reduces the growth rate of the gizzard (Romanoff, unpublished).

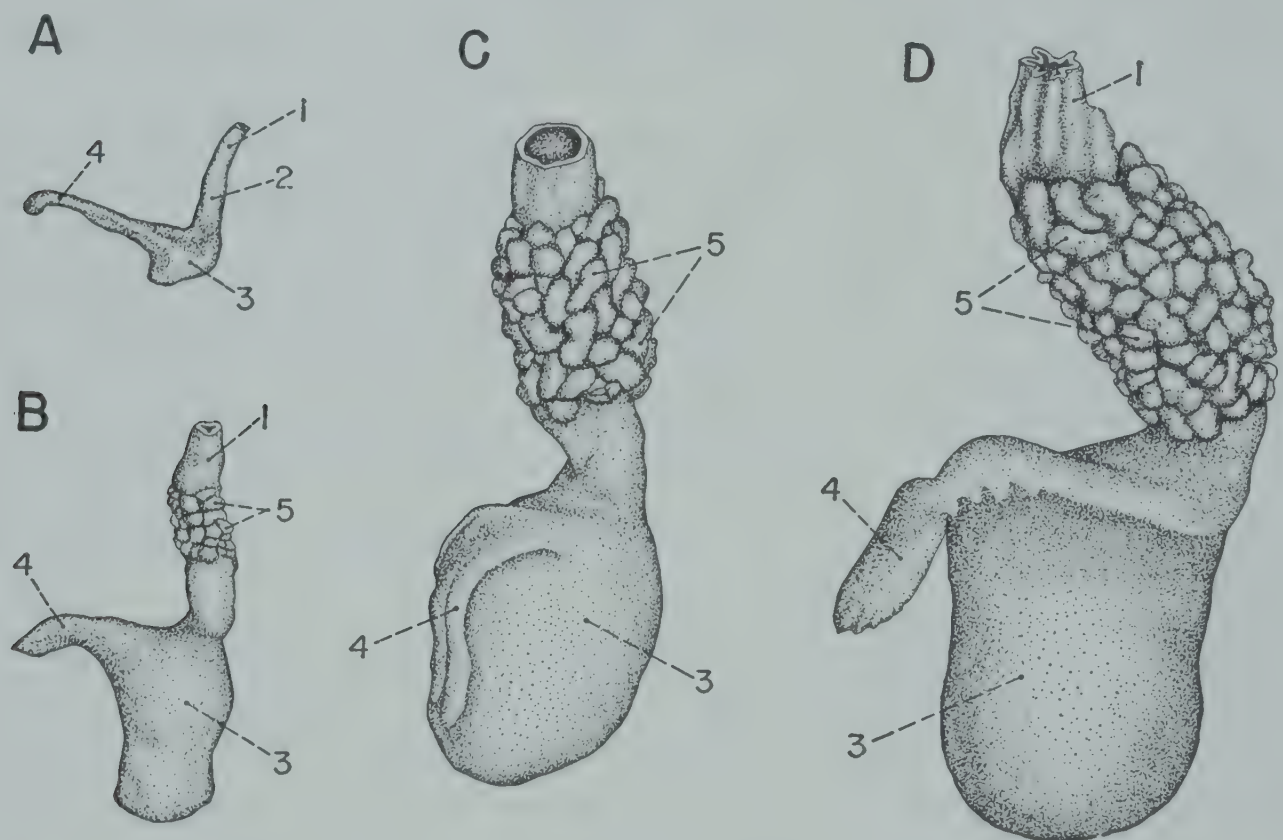


Fig. 185. Reconstruction of the endodermal portion of the stomach (proventriculus and gizzard) of the chick embryo, at various stages of development; exterior views, seen from the ventral side. (Redrawn with modifications after Sjögren, 1941.)

A, at 6 days; B, at 7 days; C, at 10 to 11 days; D, at 11 days. All  $\times 10$ .

1, esophagus; 2, proventriculus; 3, gizzard; 4, duodenum; 5, glands of the proventriculus.

The length of the chick's entire stomach complex (proventriculus and gizzard together) increases by about 50 per cent (from 1.8 cm. to 2.8 cm.) during the last week of incubation (Kuo and Shen, 1936). At hatching, the proventriculus is 1.4 cm. long in male chicks and slightly longer (1.7 cm.) in female chicks (Fronda and Marcelo, 1938), and hence accounts for approximately half the total length of the stomach.

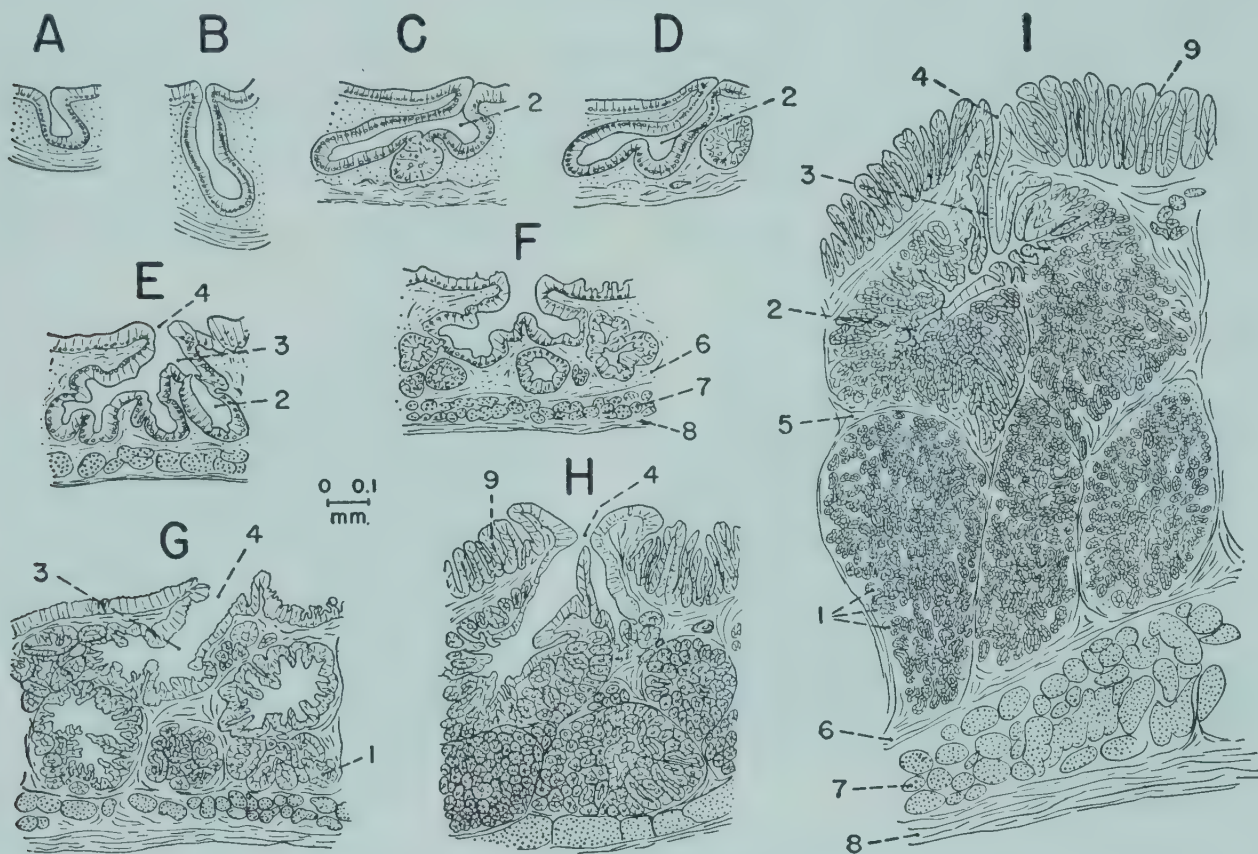
### Histological Development of the Proventriculus

The proventriculus, although composed of the same tissue layers as the esophagus, is distinguished from the latter by the abundance of glands that develop. The glands secreting digestive enzymes are simple in some birds and compound in others. They are derived from the endodermal epithelium



but lie embedded in the connective tissue, which forms the thickest portion of the proventricular wall. Superficial to the digestive glands are the tubular glands.

Development of the compound glands has been studied in detail in embryos of the chick (*Sjögren, 1941; Hibbard, 1942*) and the zebra parakeet, *Melopsittacus undulatus* (*Joos, 1941*); in the two species, it begins at approximately the same age and proceeds at almost identical rates. On the sixth or seventh day, or possibly, in the chick, on the fifth day (*Cazin,*



**Fig. 186.** Semidiagrammatic representation of successive stages in the structural and cytological development of a digestive gland of the proventriculus of the embryo of the chick, *Gallus gallus*. (Redrawn from Hibbard, 1942.)

A, at 7 days, the simple lumen; B, at 9 days, the lumen enlarges; C, at 10 days, secondary compartments appear; D, E, at 11 and 12 days, respectively, secondary compartments develop extensions; F, at 13 days, extensions subdivide; G, H, at 14 and 18 days, respectively, the parenchyma of the gland becomes more extensive; I, at hatching, the gland is composed of an extensive system of secreting tubules, emptying their secretions into secondary tubules, then into primary tubules, and then through the duct of the glands into the opening of the proventriculus, itself. All in scale.

1, alveolus of gland; 2, secondary duct of gland; 3, primary duct of gland; 4, orifice of gland; 5, submucosa; 6, muscularis mucosae of proventriculus; 7, circular muscle; 8, longitudinal muscle; 9, mucous gland.

1887; Hibbard, 1942), the semistratified cylindrical epithelium lining the proventriculus becomes folded as the result of rapid cellular division (Fig. 186-A). The folds soon appear as small, saccular, narrow-necked invaginations (Fig. 186-B) whose blind ends project into the surrounding mesenchyme (Fig. 187-A and B). According to Sjögren (1941), there are about sixty-five of these invaginations in the chick embryo. During the next 2 or 3 days of incubation, they elongate and assume a tubular form. Their de-



velopment is at this stage in the tufted penguin (*Eudyptes chrysolophus*) after 9 to 11 days of incubation (Bartram, 1901), when the compound glands consist of simple sacs measuring about 80 by 220 microns and formed of a single layer of cylindrical cells 27 microns high (or lower than the cells lining the proventriculus lumen).

By the end of the chick's eleventh incubation day, the blind ends of the glands are directed craniad (cf. Fig. 185-C), apparently because the endodermal portion of the proventriculus has elongated more than the mesenchymatous portion (Sjögren, 1941). Also the glands have given off buds basally, so that each gland now consists of two vesicles. Each vesicle in turn buds basally (Fig. 186-C, D, and E), thus doubling the number of vesicles; the eight-vesicular stage is reached by the end of the fourteenth day (Sjögren, 1941). In the parakeet (*Melopsittacus undulatus*), however,

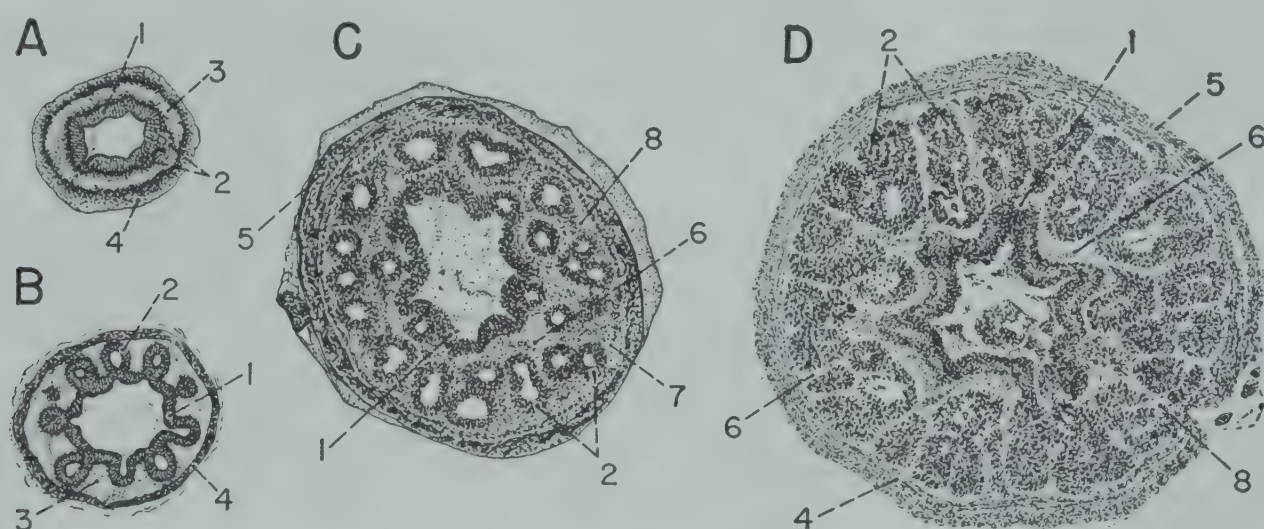


Fig. 187. Stages in the development of the proventriculus in the chick embryo, shown in cross section. (Redrawn with modification after Sjögren, 1941.)

A, at 7 days' incubation; B, at 8.5 days; C, at 11 days; D, at 14 days. All  $\times 15$ .

1, endodermal epithelial lining; 2, gland primordia; 3, mesenchyme; 4, circular muscle layer; 5, longitudinal muscle layer; 6, muscularis mucosae; 7, submucosa; 8, tunica propria.

the complexity of the gland has been seen to increase through the formation of secondary folds (Joos, 1941). The glandular epithelium decreases from cylindrical to cuboidal as the glands become multilobular.

On and after the eleventh day, the compound glands extend throughout most of the tunica propria (Fig. 187-C). At levels where the compound glands are present, the muscularis mucosae has now divided into two layers, one external to the glands, the other between and internal to them (Fig. 187-D). After the fourteenth day, the principal change in the compound glands is a rapid increase in the number of secondary lobules or saccules. These elongate, become tubular, and form the true secretory portion of the glands. Eventually, the original saccule represents the main duct of each glandular complex and the secondary saccules constitute the secondary ducts or central collecting cavities. The complexity of the compound glands



continues to increase up to the time of hatching and even afterward (Dawson and Moyer, 1948) (cf. Fig. 188- $D_1$  and  $D_2$ ).

The superficial glands lining the lumen of the proventriculus begin to appear in the lower part of the glandular stomach on the chick's eleventh

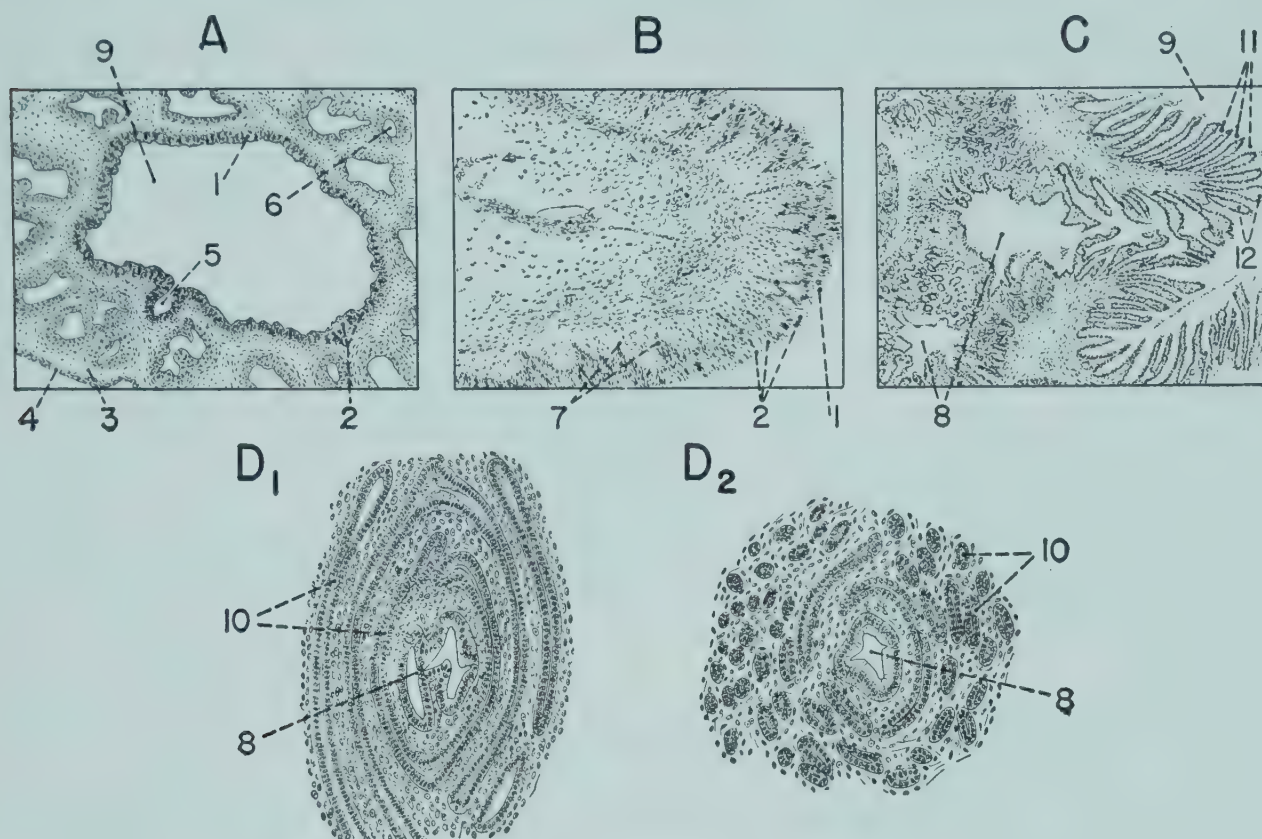


Fig. 188. Structural changes in the wall of the proventriculus of the developing chick embryo and structure of a single gland in the proventriculus of the mew gull (*Larus canus*). (Redrawn with modifications A, B, and C, after Dawson and Moyer, 1948;  $D_1$  and  $D_2$ , after Schreiner, 1900.)

A, cross section of the proventriculus of the 13-day chick embryo showing the primary and secondary sacculations characteristic of early stages in compound digestive gland development ( $\times 25$ ); B, cross section through a single fold of the mucosa of the proventriculus of the 17-day chick embryo showing the slender epithelial extensions penetrating the underlying tunica propria which represent early stages in the formation of definitive plicae and sulci. Argentophile cells are few in number and irregularly arranged ( $\times 80$ ); C, cross section of a portion of the proventriculus of the newly hatched chick showing the superficial epithelium comprised of definitive plicae and sulci and the prevalence of compound glands. No argentophile cells are distinguishable at this magnification ( $\times 25$ );  $D_1$ , superficial and  $D_2$ , deeper sections of a compound gland cut parallel to the surface of the proventriculus of the mew gull at hatching, showing the complex arrangement of ducts and septa. The excretory duct is located at the center of each section ( $\times 90$ ).

1, lining epithelium; 2, argentophile cells; 3, inner longitudinal muscle tunic; 4, circular muscle tunic; 5, primary sacculations; 6, secondary sacculations; 7, epithelial extensions; 8, duct of compound gland; 9, lumen of proventriculus; 10, tubules of compound gland; 11, sulci; 12, superficial plicae.

day and in the upper part on the thirteenth day (Sjögren, 1941). Their formation seems to be relatively later in the tufted penguin (*Eudyptes chrysolophus*), for Bartram (1901) observed that they are not yet present on the twenty-fifth day of the 35-day incubation period. The first step in



their development is active division of the epithelial cells, resulting in the formation of folds between the openings of the compound glands. By the sixteenth day of incubation, the folds are as prominent as those of the esophagus. At this time, gland anlagen begin to appear in the form of local downgrowths of epithelial cells into the tunica propria (Fig. 188-B), and gland formation now proceeds as in the esophagus. The glands eventually assume the appearance of tall, thin plicae covered with a simple columnar epithelium (Fig. 186-H and I; Fig. 188-C) and arranged concentrically around the elevated openings of the compound glands (*Dawson and Moyer, 1948*).

Granular, argentophilic cells appear in the superficial epithelium of the proventriculus on the chick's eighth day of incubation and increase in frequency until the thirteenth day (Fig. 188-A). After the sixteenth day they gradually disappear. In the compound glands, argentophile cells may be seen on the twelfth day. They increase rapidly in number, especially at the periphery of the glands, and persist after hatching (*Dawson and Moyer, 1948*).

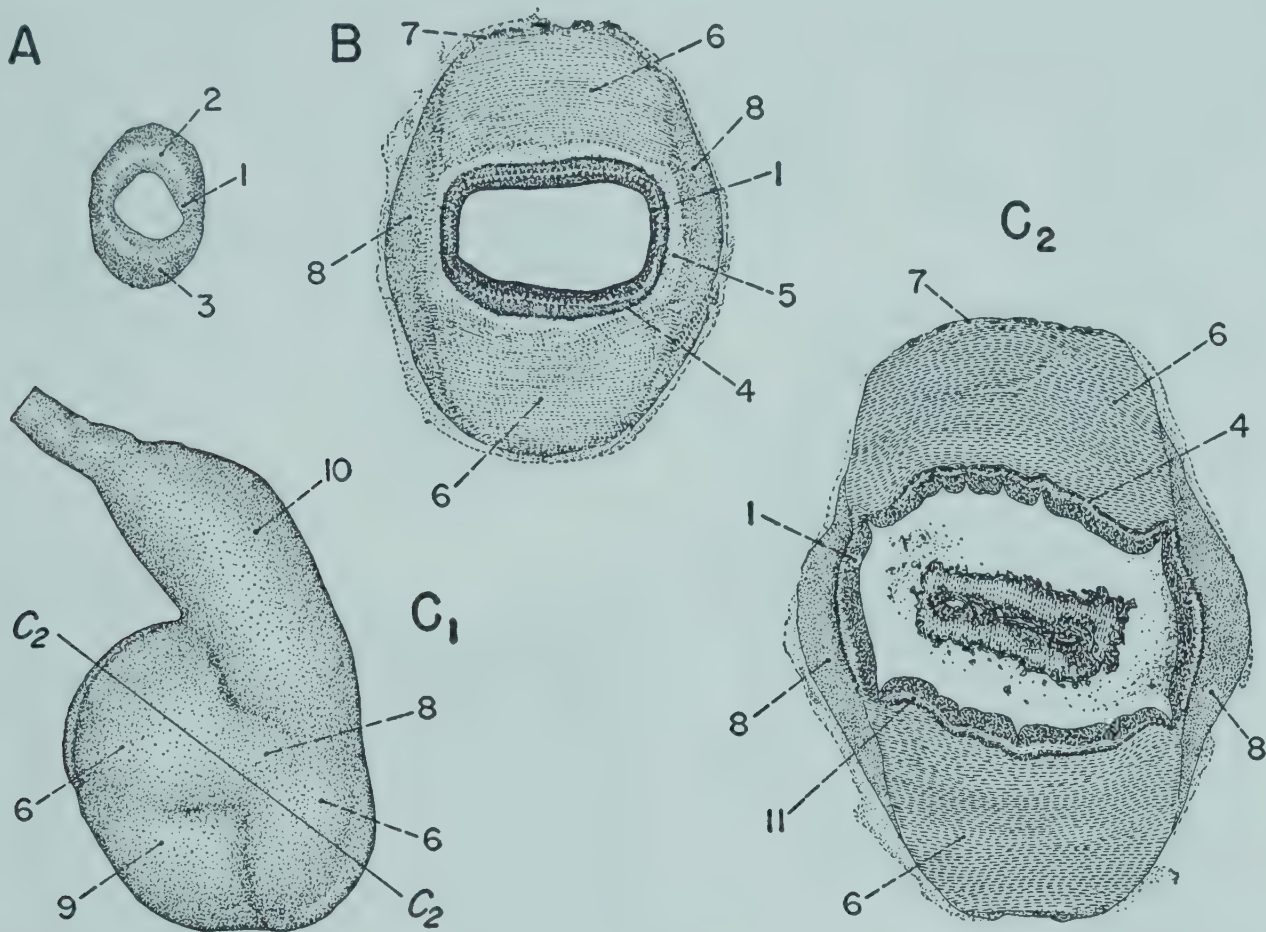
### Histological Development of the Gizzard

The gizzard is outstandingly different from the remainder of the digestive tract because the glandular and mucosal layers are relatively thin and the circular musculature is massively developed. The overwhelmingly muscular nature of the gizzard is foreshadowed on the chick's seventh incubation day, when the circular muscle layer already forms the thickest part of the gizzard wall (Fig. 189-A). The muscularis mucosae has begun to differentiate next to the endoderm. The longitudinal muscle layer, which never becomes very thick, is recognizable midway through the ninth day. On the eleventh day, the muscles of the circular layer have formed a pair of thick, semicircular bundles known as the *musculi laterales* (Fig. 189-B), although the gizzard has now rotated so that these bundles are respectively more dorsal and ventral than lateral in position. On the two lateral (formerly dorsal and ventral) surfaces of the gizzard, collagen fibers have differentiated; these fibers are the anlagen of the tendinous plates that connect the dorsal and ventral *musculi laterales*. Later in the same day, the *musculi intermedii* become apparent; these lie at the cranial and caudal ends of the gizzard and are thinner than the *musculi laterales*. The tunica propria is a thin, dense layer, and the submucosa is inconspicuous. The cavity of the gizzard is rectangular; its long diameter coincides with the short diameter of the gizzard wall. The subsequent development of the mesodermal portion of the gizzard (Fig. 189-C<sub>1</sub> and C<sub>2</sub>) consists chiefly of a continued increase in the size of its muscles (*Sjögren, 1941*).

The tubular glands lining the gizzard differ in their development from the tubular glands in other portions of the digestive tract. Joos (1952),



from observation of embryos of the chicken (*Gallus gallus*), zebra parakeet (*Melopsittacus undulatus*), pigeon (*Columba livia*), European quail (*Coturnix coturnix*), starling (*Sturnus vulgaris*), and blackbird (ouzel), *Turdus merula*, concluded that there are four distinct stages in the differentiation of typical glands secreting the characteristic keratinoid lining of the gizzard.



**Fig. 189.** Stages in the development of the gizzard in the chick embryo, shown in cross section and external view. (Redrawn with modifications after Sjögren, 1941.)

A, at 7 days' incubation ( $\times 10$ ); B, at 11 days ( $\times 10$ ); C<sub>1</sub> ( $\times 3.5$ ) and C<sub>2</sub>, at 14 days ( $\times 10$ ).

1, epithelial lining; 2, mesenchyme; 3, circular muscle layer; 4, muscularis mucosae; 5, submucosa; 6, lateral muscles; 7, longitudinal muscle layer; 8, facies tendinea; 9, intermediate muscle; 10, proventriculus; 11, tunica propria.

In the first stage, lasting from the chick's fifth to ninth (Fig. 190-A, B, C<sub>1</sub>, and C<sub>2</sub>) incubation days, inclusive, the high cylindrical epithelial cells lining the gizzard multiply so that the two or three rows of nuclei increase to four or five. At the free borders of the cells, short, delicate processes extend out into the lumen of the gizzard (Cazin, 1885; Cornselius, 1925; Hibbard, 1942). After the seventh day, these processes are covered by a thin layer of substance that is stained red by mucicarmine, although the epithelial cells themselves give no reaction at this time except for a very faint pink color confined to their distal ends.

During the second stage (Fig. 190-D<sub>1</sub> and D<sub>2</sub>), lasting for only 2 days (the tenth and eleventh in the chick, the seventh and eighth in the starling, *Sturnus vulgaris*, and blackbird, *Turdus merula*), the cell processes and the layer of secretion increase in height, and both stain red with



mucicarmine; so also do the epithelial cells. Granules have appeared at the base of the extracellular processes and form a dark line delimiting the cells (Cornselius, 1925; Joos, 1952). The epithelial cells contain an increasing number of red-stained inclusions (Fig. 191-A). The inclusions occupy the middle of the epithelial layer. A few nuclei begin to migrate into the lumen of the gizzard. Mitoses, formerly most numerous near the lumen, now become most frequent close to the connective tissue.

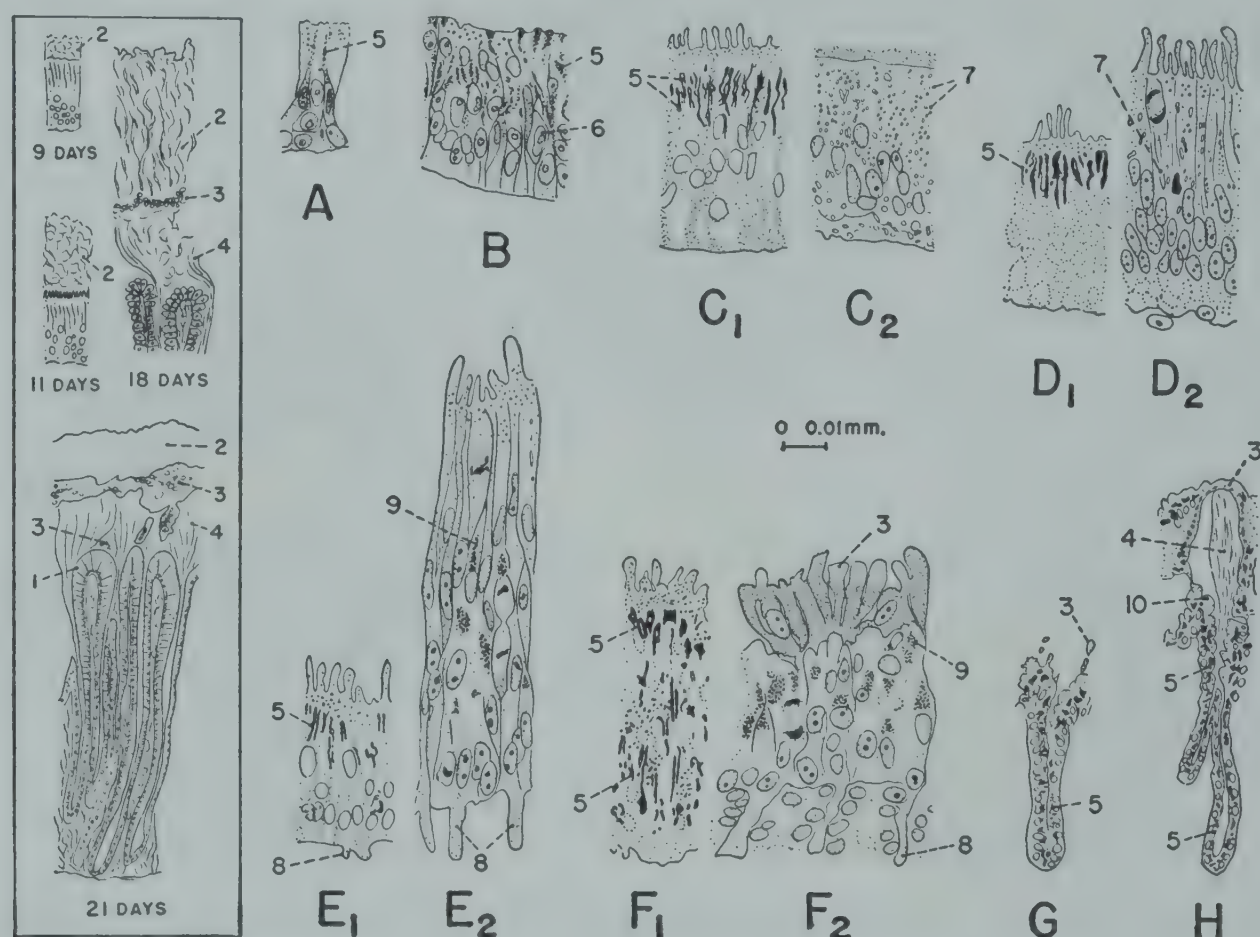


Fig. 190. Developmental changes in the lining and epithelium of the gizzard in the chick embryo. (Redrawn with rearrangement after Hibbard, 1942.)

A, at 5 days' incubation; B, at 7 days; C<sub>1</sub> and C<sub>2</sub>, at 9 days; D<sub>1</sub> and D<sub>2</sub>, at 11 days; E<sub>1</sub> and E<sub>2</sub>, at 13 days; F<sub>1</sub> and F<sub>2</sub>, at 15 days; G, at 17 days; H, at 19 days. All in scale.

Insert: schematic representation of the general changes in appearance of the lining of the chick embryo gizzard.

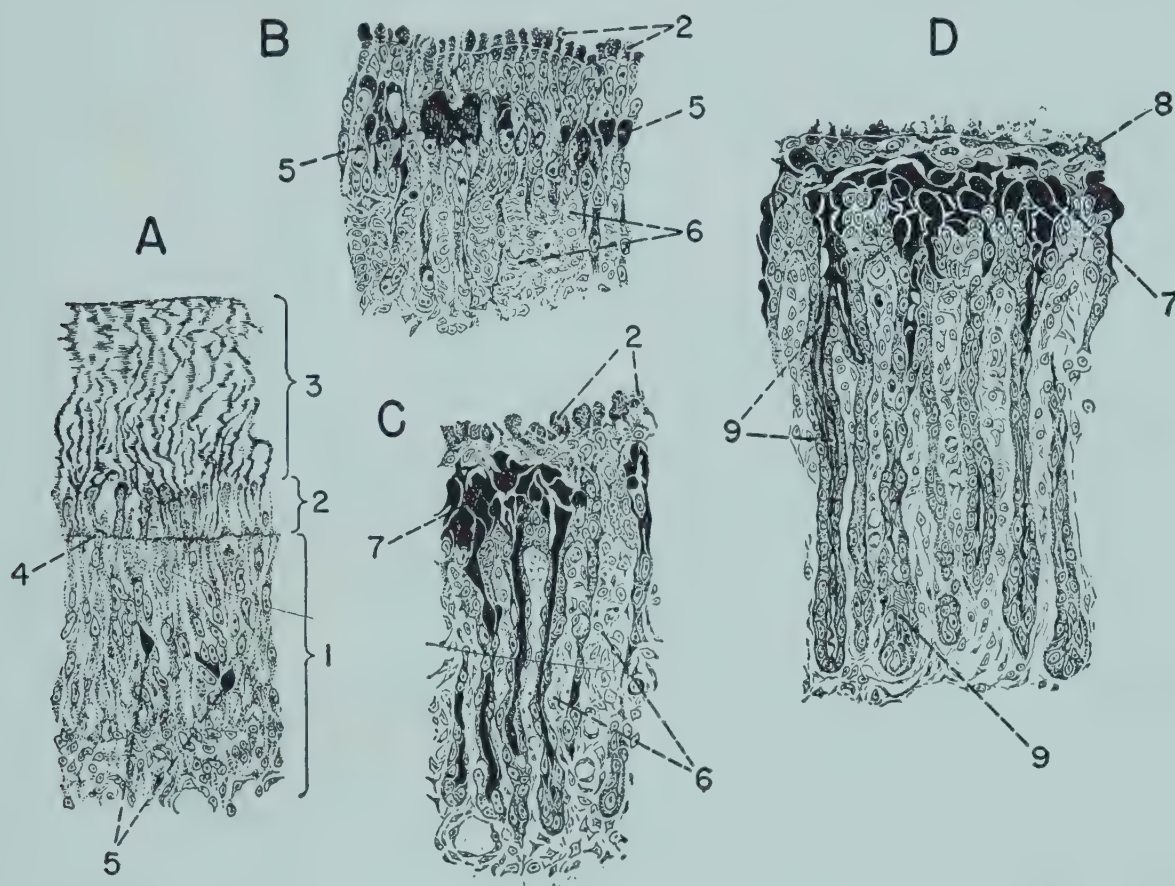
Cytological changes in the epithelium are shown in figures C<sub>2</sub>, D<sub>2</sub>, E<sub>2</sub>, and F<sub>2</sub>, by sections stained by the Helly method to show vacuoles; the remaining figures are of sections prepared by the DeFano silver nitrate method to show the Golgi apparatus.

1, epithelial cells lining the gizzard; 2, original mucous secretion; 3, cellular debris; 4, keratinoid secretion; 5, Golgi apparatus; 6, nucleus; 7, vacuole; 8, gland sprouts; 9, keratohyalin granules; 10, mucus.

The first steps leading directly to gland formation occur on the chick's twelfth day of incubation (equivalent to the ninth day, in the blackbird, *Turdus merula*, and starling, *Sturnus vulgaris*), when newly formed epithelial nuclei begin to push down into the underlying connective tissue. The cell processes projecting into the gizzard lumen are now large and club-shaped (Fig. 191-B). Soon the processes decrease in size and stain brown with mucicarmine, rather than red; eventually they are sloughed off



(Hibbard, 1942). As the penetration of epithelial cells into the connective tissue continues, sprouts and then long strands of cells are formed beneath the zone of increasingly large cellular inclusions. The cells forming these strands are not cylindrical but cuboidal (Joos, 1941). Secreted material now accumulates between the cells and forces itself up toward the lumen of the gizzard (Fig. 191-C), thus opening up lumina in the solid sprouts (Hibbard, 1942), which therefore assume the appearance of tubular glands. The cells above the zone of cellular inclusions (that is, next to the gizzard lumen) degenerate (Fig. 190-E<sub>1</sub>, E<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, and G).



**Fig. 191.** Stages in the development of glands of the gizzard lining in the chick embryo. (Redrawn with modifications after Joos, 1952.)

A, at 11 days' incubation; B, at 13.5 days; C, at 17 days; D, at 18 days.

1, epithelium; 2, extracellular processes; 3, layer of secretion; 4, dark line of granules; 5, cellular inclusions of mucin; 6, strands of cells; 7, layer of mucin formed by confluence of cellular inclusions; 8, degenerating cells; 9, secretions of keratinoid substance (indicated by crosshatching).

The final stage starts on the chick's eighteenth day (Fig. 190-H), when the secretion of a keratinoid substance begins deep in the fundi of the glands. The degenerated cells next to the lumen have been lifted away by the stream of mucin that has poured out and covered the mouths of the glands and the folds between them (Fig. 191-D). On the next day, the glands are producing an exclusively keratinoid secretion which forms a distinct stratum beneath the mucinous layer and the layer of cell debris (Hibbard, 1942). On the last 2 days of incubation, the cells between the gland mouths and facing directly on the gizzard lumen begin anew to secrete mucin.



The increase in the thickness of the gizzard lining between the chick's seventh and twenty-first day of incubation is given in the tabulation (*Hibbard, 1942*). Part of the increase, which is greatly accelerated at the close of incubation, is due to folding, and part to actual growth. Immediately after hatching, there is a rapid increase in the thickness of the keratinoid layer, which contains many cell fragments (*Cornselius, 1925; Hibbard, 1942*).

Incubation Age (days)	Thickness of Epithelium (microns)
7-13	60-65
14	100
18	130
19	150
20	180
21	260

A few argentaffin cells have been seen in the epithelium of the prepyloric region of the gizzard early in the chick's twelfth day of incubation (*Simard and Campenhout, 1932*). Argentophile cells are present in the glandular epithelium of the gizzard on the eighteenth day of incubation. They occur in the fundi or walls of the glands and not among the mucin-producing cells of the free surface. There is little increase in their relative number up to the time of hatching (*Dawson and Moyer, 1948*).

### Functional Development of the Stomach

Peristaltic contractions of the chick embryo's entire stomach complex (proventriculus and gizzard) have been seen on the eighth and ninth days of incubation (*Kuo and Shen, 1936*), that is, immediately after the differentiation of the circular and outer longitudinal muscle layers in the gizzard. The stomach of embryos 15 or more days old continues to contract when removed to Tyrode's solution. The tracings reproduced in Fig. 192 show that the stomach contracts slowly and rhythmically in the characteristic manner of smooth muscle. The frequency of contraction (from 1.5 to 2.0 per minute) is rather independent of age between the fifteenth and twenty-first days of incubation, but the amplitude of the contraction wave increases from about 3 mm. to 15 or 16 mm. during this period. The type of contraction also changes somewhat with age. At first, the stronger contractions are preceded or followed by one or two weaker contractions. By the nineteenth day, contractions are such that tracings show a small wave superimposed on either the ascending or descending limb of each large wave. The weaker contractions gradually disappear during the last 2 days of incubation. In the newly hatched chick, the contractions of the stomach are regular and of uniform strength.



The contents of the stomach increase in amount as the embryo begins to swallow amniotic fluid and then albumen (see Chapter 13). The increase becomes apparent after the eighth or ninth incubation day in the chick (Kuo, 1932a; Shklyar, 1938) and after the tenth day in the duck



Fig. 192. Kymographic records of stomach contractions of chick embryos from 15 to 22 days' incubation. (Reconstructed from tracings by Kuo and Shen, 1936.)

(*Anas platyrhynchos*) and goose, *Anser anser* (Shklyar, 1938). The tabulated figures give the amount of the "gastric contents" as determined by Kuo (1932a), who apparently weighed the contents of crop and stomach together.

Incubation Age (days)	Amount of Gastric Contents (gm.)
9	0.01
10	0.02
11	0.03
12	0.04
13	0.06
14	0.08
15	0.15
16	0.26
17	0.30
18	0.39
19	0.58
20	0.59



Evidence of secretory activity of the digestive glands is provided by the change in the reaction of the stomach contents from neutral to acid during the course of incubation. The pH has attained a value of 4.2 on the chick's twentieth day and a value of 3.15 on the goose's (*Anser anser*) twenty-seventh day. The acidity is due to the presence of free hydrochloric acid, which is presumably secreted by the glands. Very small quantities of pepsin and rennin are present in the goose's stomach during the last days of development (Shklyar, 1938). Although Kuo (1932a) could not find pepsin in the chick's stomach until the sixteenth day of incubation, other investigators (Pokorna, Haskova, and Hinzova, 1955) have stated that it is present on and after the twelfth day. The digestion of swallowed albumen would seem to require the mediation of proteolytic enzyme.

### THE SMALL AND THE LARGE INTESTINE

In birds, the intestine consists essentially of three successive divisions and therefore resembles the reptilian intestinal tract more than the mammalian. The most anterior division, leading from the gizzard, is known as the duodenum but is not strictly comparable to the structure of the same name in mammals. The duodenum is doubled upon itself in an elongated loop, with most of the pancreas enclosed between its limbs. The next and longest division of the intestine, variously termed Meckel's tract, jejunum, ileum, and jejuno-ileum, is looped and convoluted to a degree that varies in different birds but is most marked in the least primitive groups. The ileum leads into the short, straight large intestine, which is a composite of colon and rectum. In the ostrich (*Struthio camelus*), however, the large intestine is twice as long as the small intestine. In the majority of avian species, the junction of the ileum with the large intestine is marked by the presence of bilateral caeca which may be quite long. On the other hand, the caeca may be rudimentary, or absent entirely, or there may be but one caecum. The large intestine terminates in an expansion, the coprodaeum; this is the most cranial of the three divisions of the cloaca and is perhaps derived in part from the embryonic cloaca as well as from the embryonic hind-gut.

#### The Gross Development of the Intestine

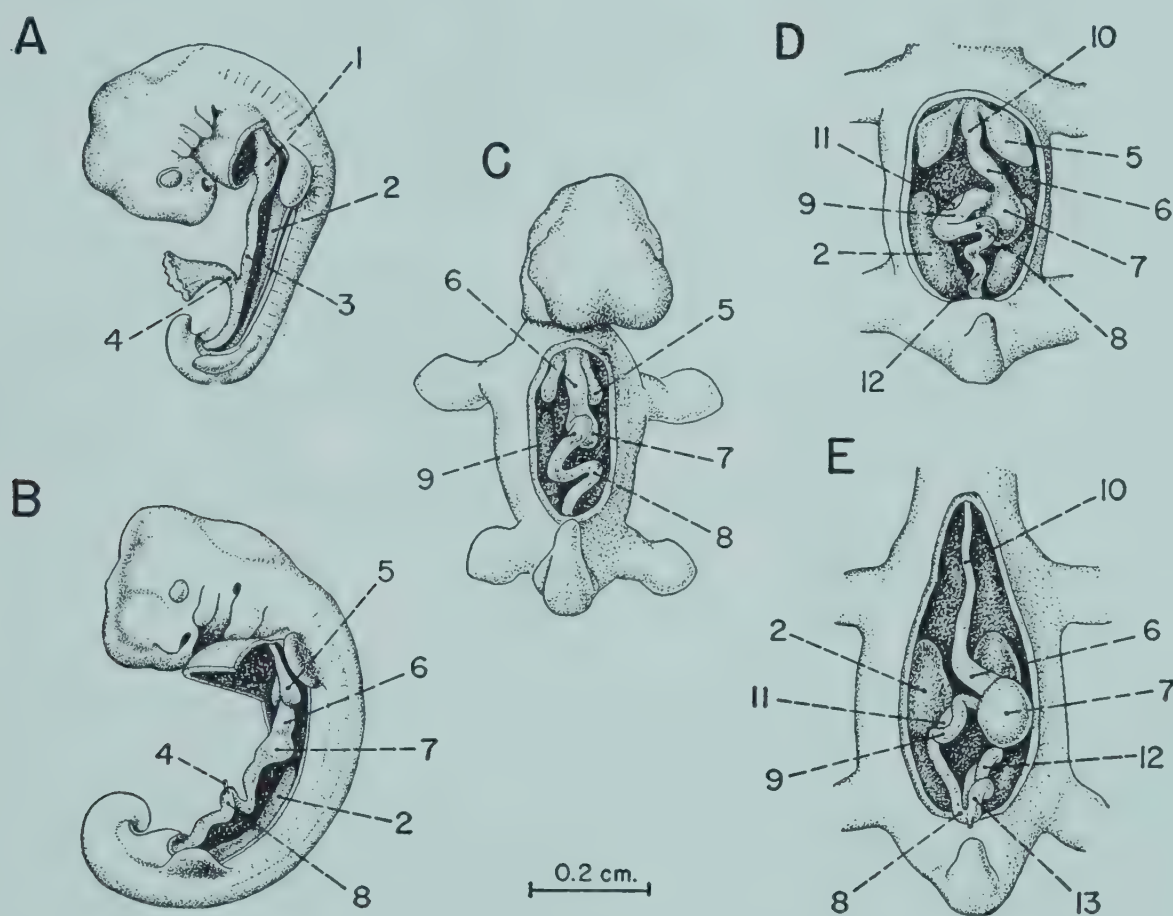
The originally straight intestinal tract develops its characteristic folds and convolutions because it elongates more rapidly than the body cavity. In all birds with incubation periods of 2 or 3 weeks, the growth of the intestine causes two loops, or curvatures, to appear almost simultaneously during the fifth day of incubation (Joos, 1941). The more anterior of these is the duodenal loop. This continues into the umbilical or primitive loop, which, with the yolk stalk depending from its apex, protrudes out of



the body cavity into the somatic umbilicus until late in the incubation period. The umbilical loop is composed entirely of mid-gut and is eventually incorporated into the ileum. The anal end of the umbilical loop is marked by the caeca (except, of course, in species lacking them), which develop from the first portion of the hind-gut that forms. The large intestine is derived from a part of the hind-gut that lies between the caeca and the cloaca but is not distinguishable until the caeca begin to differentiate.

### *The Duodenum*

The duodenal loop becomes recognizable when the gut, after a short ventrad course from the gizzard, bends sharply caudad and then obliquely



**Fig. 193.** The initial development of intestinal loops in the embryo of the zebra parakeet, *Melopsittacus undulatus*. (Redrawn with modifications after Joos, 1941.)

A and B, embryo during second half of the fifth incubation day; C, at the end of the fifth day; D, a 6-day embryo; E, a 7-day embryo. All in scale.

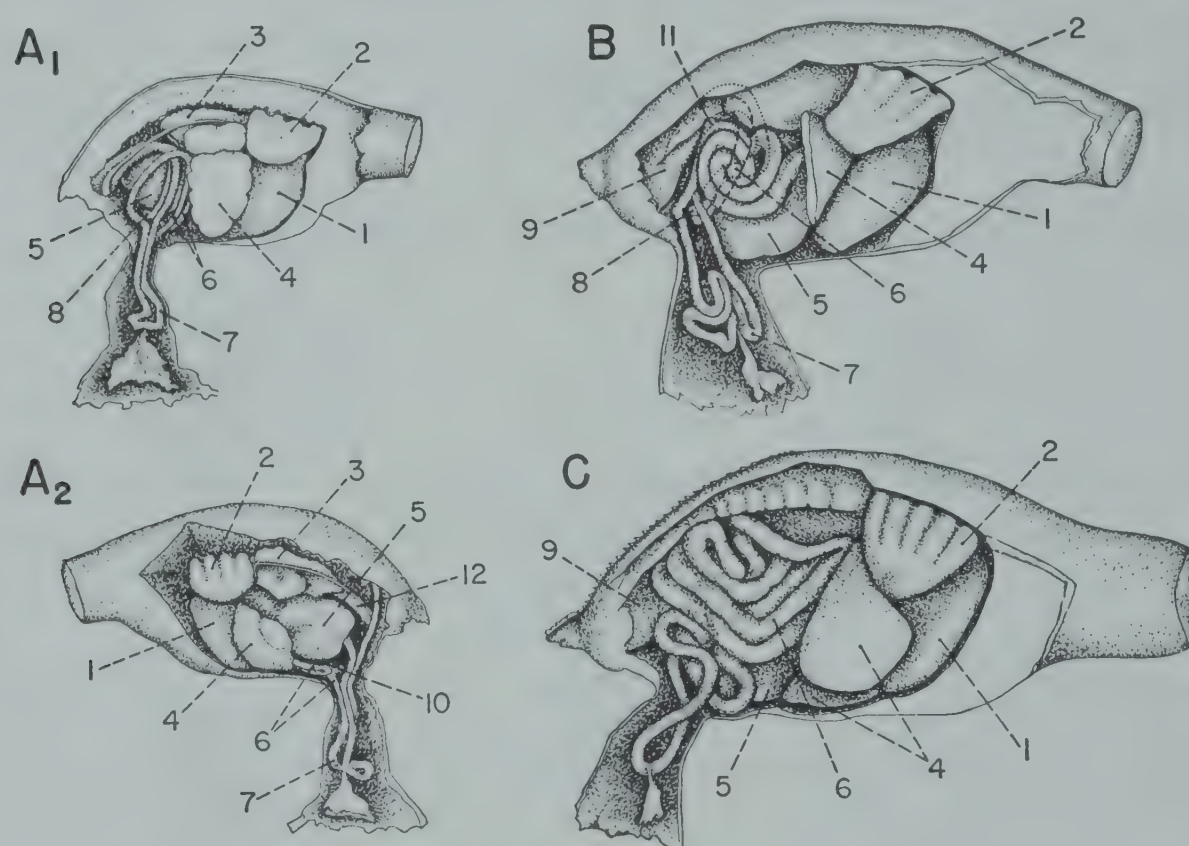
1, stomach complex; 2, kidney; 3, fore-gut; 4, yolk stalk; 5, lung; 6, proventriculus; 7, gizzard; 8, umbilical loop of gut; 9, duodenal loop; 10, esophagus; 11, pancreas; 12, supraduodenal loop, or fourth loop of ileum; 13, third loop of ileum.

to the right and slightly dorsal before turning caudad again into the umbilical loop (Fig. 193-C). In the chick, this stage may be reached at the beginning (*Brouha, 1898b*) or middle (*Kersten, 1912*) of the fifth day of incubation. The flexure between the duodenal and umbilical loops marks the boundary between the duodenum and the ileum and corresponds to the duodenojejunal flexure of mammals. The ileoduodenal flexure is con-



siderably analward of the bile duct anlagen, which mark the oral end of the mid-gut. Hence it is clear that the terminal part of the chick's duodenum is derived from the mid-gut (*Kersten, 1912*).

As the duodenum grows, the angle disappears between the ventrally and caudally directed segments, which thus become confluent and form the descending limb of the duodenal loop, running ventrad and somewhat to the left. The ascending limb is the portion coursing from left to right and dorsad. During the sixth day, the elongation of the duodenum brings the apex of the loop continually closer to the ventral body wall (Fig. 193-D).



**Fig. 194.** The later development of intestinal loops in the chicken embryo. (Redrawn with modifications after *Kersten, 1912*.)

A<sub>1</sub>, embryo incubated 11 days 21 hours, with right body wall removed; A<sub>2</sub>, embryo of same age, with left body wall removed; B, embryo incubated 12 days 22 hours, with right body wall removed; C, embryo incubated 15 days 22 hours, with right body wall removed. All  $\times 1.5$ .

1, heart; 2, lung; 3, kidney; 4, liver; 5, gizzard; 6, duodenum; 7, ascending limb of umbilical loop; 8, right caecum; 9, cloaca; 10, left caecum; 11, ileum; 12, large intestine.

By the middle of the seventh day, the ascending limb runs directly ventrodorsad instead of transversely (*Brouha, 1898b*). Close to the end of the seventh day, the pars pylorica of the duodenum begins to set off clearly from the gizzard. The apex of the duodenal loop is now directed caudad, perhaps under the influence of the growth of the pancreas, which fills the space between the ascending and descending limbs (Fig. 193-E). The ends of the loop are separated by the ductus venosus. The ascending limb, to the right of the descending limb, courses slightly to the right and lies in a groove on the median surface of the liver. About 24 hours later, the pars pylorica is completely delimited from the gizzard. The ends of the duo-



denal loop are now close together. The descending limb is straight, and its course is almost perpendicularly ventrad. The apex of the loop is very close to the ventral body wall. The ascending limb lies almost caudal to the descending limb. During the next 2 days, the growth of the intestine in the region of the ileoduodenal flexure causes the ascending limb to rotate to the right until it again lies wholly on the right side of the descending limb. Between the tenth day and the end of the twelfth day, the apex of the duodenal loop grows caudad and slightly to the left along the body wall (Fig. 194-A). On the thirteenth day, the rapid elongation of the descending limb causes the duodenal loop to roll into a spiral on the right side of the gizzard, with the descending limb forming the outer curvature of the spiral (Fig. 194-B). The spiral, however, is soon forced to unwind as the growth rate of the ascending limb begins to match that of the descending limb. By the end of the sixteenth day of incubation, the two limbs of the duodenal loop lie close together in the right half of the body cavity. With the descending limb ventral to the ascending, they follow a course that is generally longitudinal but shows a slight ventral curvature (Fig. 194-C). The apex of the loop curves around the caudal edge of the gizzard to its left side. The only subsequent change in the position of the duodenal loop is a shift closer to the ventral body wall (Kersten, 1912). At hatching, the duodenum weighs 0.2 gm. (Fronza and Marcelo, 1938).

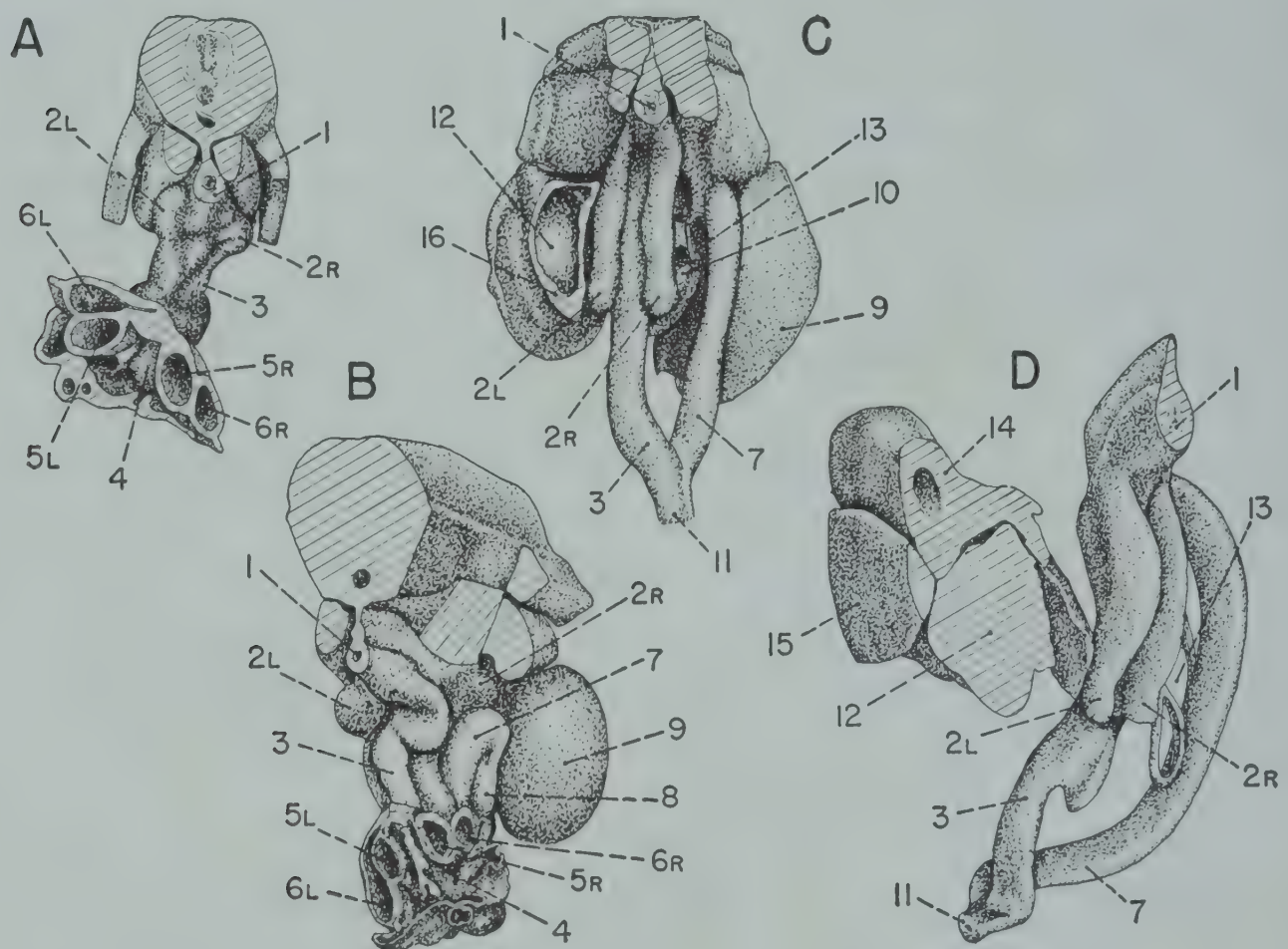
#### *The Umbilical Loop and the Ileum*

The umbilical loop begins as a slight ventral curvature of the intestine at the level where the narrow yolk stalk is given off (Fig. 193-B). As the loop protrudes farther, it can be seen that it is not quite in the sagittal plane, for its descending limb occupies the right anterior one fourth of the umbilical cord and its ascending limb occupies the left posterior one fourth. The apex of the loop is directed slightly caudad. By the middle of the chick's sixth day of incubation (Fig. 195-B), it is apparent that the descending limb has begun the process of rotation to the right and caudad (Fig. 195-C and D) that terminates at the end of the eleventh day with the two limbs reversed in their positions relative to each other (Kersten, 1912). This rotation does not occur in the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941). In the chick, the continually elongating umbilical loop remains simple in form until the end of the twelfth day, when a double curvature appears in the ascending limb (cf. Fig. 194-A). By the end of the fourteenth day, the loop protrudes 1 cm. from the body cavity (Kersten, 1912). There is now a secondary curvature in the descending limb, and the convolutions of the ascending limb are more numerous. The retraction of the umbilical loop into the body cavity (cf. Fig. 194-C) begins on the sixteenth or seventeenth day (Duval, 1889; Kersten, 1912).



and ends on the nineteenth day when the yolk stalk (Meckel's diverticulum) is drawn in (see Chapter 13).

The umbilical loop, being part of the ileum, divides the latter into a preumbilical and a postumbilical portion. In the zebra parakeet (*Melopsittacus undulatus*), the apex of the umbilical loop persists as the second, in craniocaudal order, of four loops that characterize the ileum. Three of these loops have a slightly spiral torsion, but the fourth or most caudal



**Fig. 195.** Early development of the umbilical loop and the caeca in the chick embryo. (Redrawn with modifications after Kersten, 1912.)

A, 4 days 20 hours, viewed from behind and slightly from below; B, 5 days 16 hours, right posterior view; C, 6 days 16 hours, posterior view; D, 7 days 23 hours, from behind and the left. All  $\times 7$ .

1, hind-gut; 2L, 2R, left and right caeca; 3, ascending limb of umbilical loop; 4, anterior intestinal portal; 5L, 5R, left and right omphalomesenteric arteries; 6L, 6R, left and right omphalomesenteric veins; 7, descending limb of umbilical loop; 8, ascending limb of duodenal loop; 9, right lobe of liver; 10, apex of duodenal loop; 11, yolk stalk; 12, stomach; 13, ductus venosus; 14, proventriculus; 15, left dorsal lobe of liver; 16, mesogastrium.

loop (often known as the supraduodenal loop) is elongated. This loop appears on the sixth day of incubation caudal to the umbilical loop (cf. Fig. 193-D). On the following day, the third loop is indicated directly posterior to the yolk stalk (cf. Fig. 193-E). The first or most cranial loop forms anterior to the yolk stalk by the end of the eighth day and begins to exhibit a slightly spiral configuration on the twelfth or thirteenth day (Joos, 1941).



In the chicken, the convolutions of the ascending and descending limbs of the umbilical loop form the most complexly undulating portion of the definitive ileum. The preumbilical ileum remains more or less straight until the twelfth or thirteenth day, when, because of its increased length, it curves caudad in a cranially open arch around the caudal edge of the gizzard. By the end of the fourteenth day, it runs almost horizontally from the angle of the ileoduodenal flexure as far as the caudal end of the kidneys where it takes a sharp turn ventralward and proceeds to the umbilicus. The postumbilical ileum has also elongated; it turns cranial between the spiraled duodenum and the gizzard, turns dorsal at the level of the ileoduodenal flexure, and then proceeds caudad between the kidneys. At the end of the incubation period, the ileum occupies the right caudal one fourth of the body cavity and weighs about 0.4 gm. (*Fronza and Marcelo, 1938*). The loops of the preumbilical ileum are dorsal to the duodenal loop and to the loops of the postumbilical ileum.

### *The Caeca*

The caeca, if present, arise at the boundary between the mid-gut and the hind-gut. In the chicken, the development of the long bilateral caeca begins at the end of the third incubation day or early in the fourth day, when the lumen of the postumbilical gut narrows near the cloaca and the navel but remains wide at an intermediate level (*Kersten, 1912*). The passively formed expansion starts to enlarge actively during the first half of the fourth day as the mesenchymal portion of its wall becomes thicker and the ventral half of its lumen dilates (*Goette, 1867; Kersten, 1912*). Since the mesenchymal thickening is of greater anterior extent than the expansion of the lumen, it appears that the mesodermal portion of the caecal walls is derived in part from the mid-gut (*Kersten, 1912*). Before the end of the fourth day, the caecal primordia become visible externally as bilateral swellings halfway between the umbilicus and the allantoic diverticulum (*Keibel and Abraham, 1900; Maumus, 1902; Forssner, 1907*). Initially—and, in fact, until the sixth day—the caeca are somewhat asymmetrical (Fig. 195-A and B), the left primordium being slightly larger and more caudally located than the right one (*Kersten, 1912*).

The subsequent development of the caeca was described by Maumus (1902) and Kersten (1912) as follows. By the middle of the fifth day, the caeca have grown out as conical projections, forming the flask-shaped enlargement noted by Remak (1855). Each contains a narrow lumen, an extension of the previously formed expansion of the gut cavity. During the second half of the fifth day, it is apparent that the apexes of the caeca are directed cranial (*Goette, 1867*). The segment of gut anterior to the cloaca now grows longer, and the caeca therefore appear to approach the umbilicus. As the umbilical loop forms, they are found at the termination



of its ascending limb. Slight dorsal and ventral grooves can be seen setting off each caecum from the gut proper.

By the end of the sixth day, the caeca are considerably larger. They each form a very acute angle with the intestine, and their apexes are free for a distance of 120 microns. The lumen of the gut proper is no longer expanded at the level where the caeca are given off. The dorsal and ventral grooves separating the caeca from the intestine have deepened, and a short, very thick mesentery (mesocaecum) has thus become recognizable on each side. On the seventh day, the caeca are 2 to 4 mm. long, cylindrical in shape, and show a slight terminal swelling (cf. Fig. 195-C). They are applied against the ascending limb of the umbilical loop and extend to the floor of the body cavity. They follow this generally dorsoventral course for the greater part of the incubation period.

TABLE 8

Length and Diameter of Different Divisions of Intestinal Tract of the Chick Embryo, *Gallus gallus* \*

Age of Embryo	Duodenum		Ileum		Large Intestines		Caecum	
	Length	Diameter	Length	Diameter	Length	Diameter	Length	Diameter
(days)	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)
5	1.5	0.30	2.7	0.22	1.2	0.19	0.95	0.16
6	2.0	0.30	3.4	0.32	1.4	0.25	1.35	0.22
7	3.6	0.34	7.0	0.30	1.5	0.37	2.1	0.23
8	4.5	0.38	12.4	0.33	1.5	0.48	3.1	0.28
9	6.5	0.45	16.3	0.35	2.1	0.50	3.3	0.32
10	8.3	0.50	20.1	0.38	4.0	0.65	4.0	0.35
11	8.5	0.75	21.5	0.58	5.2	1.0	4.2	0.50
12	13.5	0.83	28.0	0.65	6.0	1.1	5.3	0.60
13	21.0	1.4	70.0	0.93	9.0	1.6	9.5	0.75
14	29.5	0.90	44.0	0.8	8.0	1.3	12.0	0.95
15	36.0	1.4	81.0	1.0	9.0	1.7	13.0	1.05
16	38.0	1.4	116.0	0.8	10.0	1.8	16.5	0.95
17	44.0	1.3	119.0	0.8	12.0	1.9	17.0	0.83
18	52.0	1.7	158.0	1.15	15.0	1.8	20.5	1.25
19	48.0	1.6		1.4			22.0	1.20
20	54.0	1.9	166.0	1.75	19.0	2.3	27.0	1.65
21	50.0	1.6	132.0	1.65	18.0	2.2	28.0	1.60

\* After Kersten (1912), with modifications.

On the seventh day, the right caecum begins to swing around to the left behind the intercaecal ileum (Fig. 195-C and D) and, by the end of the ninth day, becomes applied against the left caecum, which has moved somewhat to the right. After the ninth day, the caeca increase in diameter,



especially throughout their distal two thirds. By the twelfth day, their largest portion is twice as thick as the intercæcal ileum. Their distal ends start to move apart from each other and to recurve sharply.

With the beginning of the third week of development, the caeca enter upon a period of accelerated growth which increases their length from about 12 mm. on the thirteenth day to 28 or 30 mm. by the twenty-first (see Table 8) when they weigh about 0.2 gm. (*Fronza and Marcelo, 1938*). During this time the tendency to separate continues, so that they return to the lateral surfaces of the ileum by the end of the seventeenth day. After the eighteenth day, they alter their dorsoventral course as their distal ends move caudalward and dorsalward. They do not assume their definitive, generally horizontal position until about 2 weeks after the hatching date.

The small caeca of the tufted penguin (*Eudyptes chrysolophus*) are seen in the embryo as inconspicuous, round protuberances (*Bartram, 1901*). Absence of caeca in adult life is apparently not an indication of their retrogression; they do not appear at all during the embryonic development of the zebra parakeet, *Melopsittacus undulatus* (*Abraham, 1901; Joos, 1941*).

### *The Large Intestine*

In the chick embryo, almost all of the hind-gut cranial to the cloaca is occupied by the caecal primordia until the end of the fourth incubation day. At this time, the segment of gut between the allantoic stalk and the caudal boundary of the caecal enlargement begins to elongate, and it is now possible to speak of a large intestine in the definitive sense (*Kersten, 1912*). By the eighth day, the large intestine is about 1.5 mm. long and extends straight forward to a level somewhat anterior to the umbilicus, and its course and relative position are little altered thereafter. Throughout incubation, it remains the shortest of the three divisions of the gut, and it is only 18 to 20 mm. long on the last day (see Table 8). In diameter, however, it is larger than the duodenum and ileum at all times after the sixth or seventh day (*Forssner, 1907; Kersten, 1912*).

The lumen of the large intestine narrows and is partially or completely lost at some time between the middle of the fifth and seventh day (*Gasser, 1880; Minot, 1900a; Forssner, 1907; Kersten, 1912*). Occlusion occurs in the caudocranial direction (*Minot, 1900a; Forssner, 1907; Kersten, 1912; Pap, 1933*) and, as in the esophagus, is due to the presence of an epithelial plug. Closure of the large intestine at a comparable developmental period has been observed in embryos of the European lapwing, *Vanellus vanellus* (*Abraham, 1901; Hafferl, 1926*). Recanalization, which has been described as proceeding both craniocaudad (*Kersten, 1912*) and caudocranial (*Pap, 1933*), is complete by the end of the chick's twelfth incubation day (*Gasser, 1880; Pap, 1933*).



### The Histological and Functional Development of the Intestine

The internal surface of the avian intestine is lined with projecting folds or villi. These are arranged in various ways in different species, but either a honeycomb or a longitudinal zigzag pattern is most frequently seen when the intestine is opened and laid out flat (Müller, 1922). The regularity of arrangement is usually most marked in the midportion of the ileum. The villi may be short, leaflike folds, or they may be conical or finger-like projections. In general, the villi decrease in height and abundance from the cranial to the caudal end of the intestine. Between them are the openings of the intestinal glands, or crypts of Lieberkühn. The histological structure of the villi and crypts is much like that found in the mammalian intestine. The cylindrical epithelium covering the villi is continuous with the epithelium of the glands; the nuclei of the cells are situated basally and their free ends are covered with a transversely striated cuticle. Mucin-secreting goblet cells are interspersed among the cylindrical cells. Argentaffin cells (chromaffin or basally granular cells) occur in the epithelium and underlying tissue. Embedded in the stroma of the villi are fibers of the muscularis mucosae, blood and lymphatic capillaries, elastic fibers, and nerves. The outer layers of the intestine are the same as those in the remainder of the alimentary canal.

#### *The Small Intestine*

The small intestine of the chick embryo is lined with simple columnar epithelium until the end of the third day of incubation (Forssner, 1907). During the fourth day, the epithelium becomes pseudostratified (Forssner, 1907; Pap, 1933). Within each epithelial cell, long chondriocents are scattered uniformly throughout the cytoplasm, parallel to the cell's long axis (Argeseanu and May, 1938). At the end of the seventh day, many of the cells nearest the lumen degenerate (Pap, 1933), with the result that the remaining nuclei are aligned in a single basal row by the eighth or ninth day (Argeseanu and May, 1938). At this time, as on the sixth day in the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941), there is a marked rise in mitotic activity. As the cells increase in number, they decrease in height (Pap, 1933; Joos, 1941), and most of the mitochondria take up a supranuclear position (Pap, 1933; Argeseanu and May, 1938).

The surrounding mesenchymal cells do not multiply as rapidly as the epithelial cells. When the circular muscle layer appears, it increases the resistance encountered by the expanding epithelium. The latter, therefore, is thrown up into longitudinal folds, into which the mesenchyme follows. In the chick, the development of the folds may start in the duodenum and progress analward (Hilton, 1902; Forssner, 1907), or it may begin in the preumbilical ileum, then occur successively in the duodenum and the



postumbilical ileum (Pap, 1933). The folds are first seen in the parakeet (*Melopsittacus undulatus*) on the sixth day (Joos, 1941) and in the chick on the eighth (Hilton, 1902; Forssner, 1907) or ninth day (Pap, 1933). They extend to the end of the small intestine on the chick's eleventh day (Hilton, 1902; Forssner, 1907).

The number of folds is usually three to five in the beginning and then increases as new folds develop between the first ones (Torikata and Shindo, 1923; Pap, 1933). In the duodenum of the chick embryo, from nine to sixteen folds have appeared by the tenth (Hilton, 1902) or twelfth day (Goette, 1867). A larger number of folds always forms in the duodenum than in the ileum (Hilton, 1902; Forssner, 1907), because the duodenum is of wider caliber; thus, in tufted penguin (*Eudyptes chrysolophus*) embryos, twelve duodenal and four ileac folds are found at 9- to 11-day stages, and sixteen duodenal and ten ileac folds at 17- to 20-day stages (Bartram, 1901).

At first broad and irregular, the folds grow narrower and straighter as they increase in number. Soon, however, their straight longitudinal course becomes undulating and finally sharply zigzag; in the chick and the zebra parakeet (*Melopsittacus undulatus*), this change occurs between the eleventh and thirteenth days (Goette, 1867; Hilton, 1902; Joos, 1941). According to Pap (1933), the formation of each angle in the fold is due to the degeneration of cells on one side, directly opposite a center of mitotic activity on the other side; the regular alternation of centers of degeneration and mitosis along each side produces the zigzag configuration (Fig. 196-A, B, and C).

The zigzag folds now begin to break up into villi, beginning in the duodenum and progressing caudad (Hilton, 1902; Pap, 1933). Segmentation occurs at the angles and separation into villi takes place from the apexes of the folds toward their bases (Hilton, 1902). In the 15-day zebra parakeet (*Melopsittacus undulatus*), the folds have been transformed into elongated, leaflike villi arranged in a zigzag pattern (Joos, 1941). In the chick, villus formation begins on the thirteenth day, and, by the fifteenth day, the villi are beginning to lose their zigzag arrangement (Hilton, 1902). At this time, cells in the mesenchymal stroma degenerate, and their degeneration stimulates the differentiation of erythrocytes. These accumulate in cavities in the upper part of the villi and cause the latter to swell and to project farther into the lumen. Pap (1933) regarded the swollen structures as the earliest true villi (Fig. 196-B). On the chick's sixteenth day, vascular tissue invades the villi as far as the cavities containing the clumps of red blood cells (Pap, 1933). The epithelial cells are now low cuboidal, and very few mitochondria remain at their basal ends (Argeseanu and May, 1938).

During the last days of development, the villi elongate (Joos, 1941; Pap,



1933). They also increase in number by division from the apex downward (Pap, 1933); eighty rows of villi may be present in the small intestine of the chick at the end of incubation (Torikata and Shindo, 1923). At this time, the epithelial cells are again approaching the columnar form (Joos, 1941).

Active mitosis in the epithelium between the villi brings about the invagination of the first crypts of Lieberkühn on the zebra parakeet's (*Melopsittacus undulatus*) fourteenth day (Joos, 1941) and the chick's

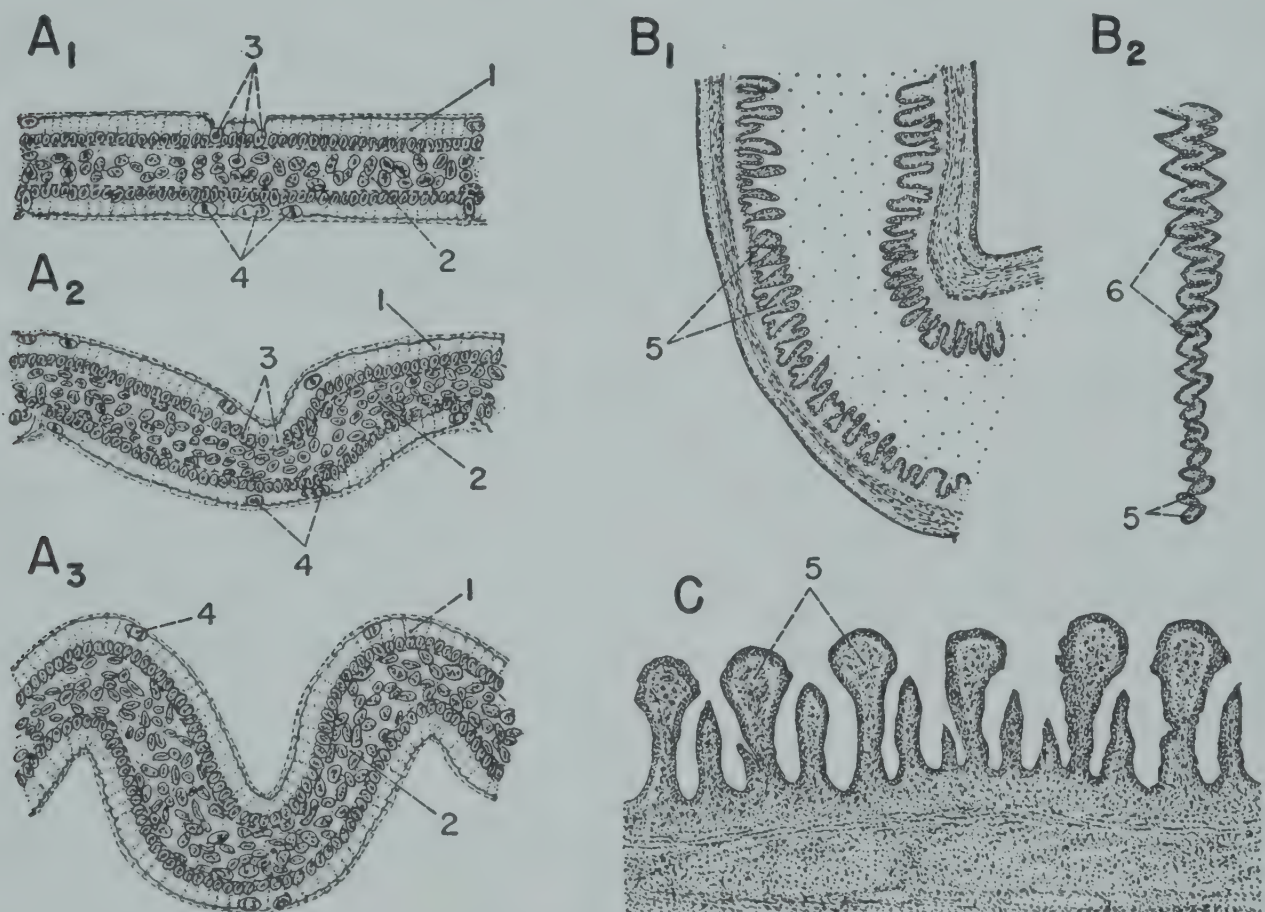


Fig. 196. The development of villi in the small intestine of the chick embryo. (Redrawn with modifications A and C, after Pap, 1933; B, after Hilton, 1902.)

A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, horizontal sections through longitudinal folds in the duodenum of 10- and 11-day embryos, showing the development of the zigzag fold from the straight fold ( $\times 300$ ); B<sub>1</sub>, longitudinal section of the small intestine of the 14-day embryo ( $\times 30$ ); B<sub>2</sub>, a slanted horizontal section through one fold, the upper end of the section being at a deeper level than the lower end ( $\times 30$ ); C, section of the ileum from a 17-day embryo, showing the club-shaped villi ( $\times 50$ ).

1, epithelium; 2, stroma; 3, degenerating cells; 4, cells in mitosis; 5, villi; 6, zigzag folds.

seventeenth (Pap, 1933). These glands, like the villi, develop first in the duodenum. The late appearance of the crypts in the tufted penguin (*Eudyptes chrysolophus*) is indicated by Bartram's (1901) failure to find them in the 25-day embryo, the oldest which he examined.

In the chick, argentaffin cells begin to differentiate directly from intestinal epithelial cells (Simard and Campenhout, 1932) at the end of the tenth (Ghidini, 1939) or eleventh day (Simard and Campenhout, 1932). They are first seen immediately adjacent to the umbilicus and then appear



at progressively more cranial and more caudal levels, so that they are present throughout the small intestine by the end of the fourteenth day; they are not numerous, however, until later. Early in the twelfth day, some of them migrate into the underlying connective tissue (*Simard and Campenhout, 1932*). These cells are present in the 12-day zebra parakeet embryo (*Melopsittacus undulatus*); they probably appear on the ninth day (*Joos, 1941*).

A few goblet cells are seen in the epithelium of the intestinal villi on the parakeet's fourteenth day (*Joos, 1941*) but, as in the chick (*Pap, 1933*), they do not appear in abundance until late in development. Round cells with eosinophilic granules (probably wandering cells) are present in the duodenal epithelium of the 15-day chick embryo, and a few lymphocytes wander into the intestinal epithelium shortly before hatching (*Pap, 1933*).

A striated border of cuticula is present at the free ends of the intestinal epithelial cells prior to the chick's seventh incubation day, but it varies in thickness until approximately the tenth day, when it acquires its definitive appearance (*Pap, 1933*). In the tufted penguin (*Eudyptes chrysolophus*) also, the cuticula is seen soon after the midpoint of the incubation period; in the zebra parakeet (*Melopsittacus undulatus*), it appears shortly before hatching, according to *Joos (1941)*. When the duodenum is in a functional state, the striated epithelial border contains alkaline phosphatase (phosphomonoesterase), whose exact role in the intestine is not clear. This enzyme has been detected histochemically in the border of the duodenal epithelium of the 12-day chick embryo, distributed in such a manner as to suggest that it assumes its marginal position in the distal limb of the duodenal loop before it does so in the proximal limb (*Hancox, 1954*). On the eighteenth day, alkaline phosphatase is histochemically demonstrable in the margins of the epithelial cells at the apex of the duodenal loop. Simultaneously, the phosphatase activity (assayed biochemically) of this part of the duodenum begins to rise very rapidly, after a slow increase starting on the ninth day (*Moog, 1950*). The injection of hydrocortisone or cortisone acetate into the egg (*Moog, 1952; Moog and Richardson, 1955*), or its presence in culture media (*Moog and Nehari, 1954*), causes alkaline phosphatase to appear precociously in the border of the duodenal epithelium, *in ovo* or *in vitro*, probably by hastening the differentiation of the cells. A nonspecific esterase also becomes increasingly concentrated in the striated border from the ninth to the nineteenth day, while also accumulating in the cytoplasm of the cells. The activity of this enzyme, determined biochemically, rises in the same manner as that of alkaline phosphatase, though not as markedly (*Richardson, Berkowitz, and Moog, 1955*).

The differentiation of the mesenchymal portion of the intestine begins in the chick on the fifth day of incubation, when the mucosa can be dis-



tinguished from the peripheral muscle strata (Gozzi, 1940), in which the as yet undifferentiated myoblasts are disposed in a circular and a longitudinal layer (Staudacher-Dalle Aste, 1941). Numerous ganglia, composed of multipolar and unipolar cells, are present near and within the muscle layer, but there are few connections between the ganglia (Staudacher-Dalle Aste, 1941). On the sixth day, short filaments appear in the muscle layers; a day later, myofibrils are abundant and are clearly seen converging toward the extremities of the myoblasts (Gozzi, 1940). In embryos 8 to 11 days old, muscular differentiation is evident. The myoblasts are elongated, with finely striated protoplasm and a sparse, granular chondriome. Some of the nuclei are vesicular and others oval. The plexus of Auerbach is present and there are fine connections between the ganglia. Most of the ganglion cells are multipolar (Staudacher-Dalle Aste, 1941).

The muscularis mucosae is present on the fourteenth day in the chick (Pap, 1933) and the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941), and before the twenty-third day in the tufted penguin (*Eudyptes chrysolophus*); in the latter bird, the outer longitudinal layer of muscle has become very thin by the time the muscularis mucosae appears (Bartram, 1901). The differentiation of the muscularis mucosae seems to begin near the umbilicus and to progress cranial and caudad from there (Simard and Campenhout, 1932). This muscle layer starts to extend into the intestinal villi on the chick's seventeenth day and reaches their apices on the last day of incubation (Pap, 1933).

Observations of excised fragments of the chick embryo's intestine held in culture media or saline solutions at 39° C. or 40° C. indicate that spontaneous contractile activity is possible as soon as mesenchymal differentiation begins, that is, before myofibrils appear. Weak and irregular local contractions have been seen in pieces of intestine from embryos incubated only 100 to 110 hours, and muscular activity already has a peristaltic character in fragments of gut from embryos 5 days old. The first rhythmic contractions each last 12 or 13 seconds and are reported three or four times a minute; the speed of the contractile wave is about 20 mm. per minute (Staudacher-Dalle Aste, 1941). It has been said that it is only explants from the most distal part of the small intestine (that is, the portion immediately proximal to the caeca) which exhibit contractility at the 5-day stage (Gozzi, 1940). Machii (1930a) noted that muscular activity first affects the caliber of the intestinal lumen, then produces corresponding changes in the diameter of the intestinal wall, and finally causes flexion and extension of an entire segment of excised gut. As embryonic age increases, contractile activity becomes more marked (Machii, 1930a; Staudacher-Dalle Aste, 1941). In explants from embryos 8 to 11 days old, contractions lasting only 5 or 6 seconds occur five to seven times per minute, and the speed of the contractile wave is 100 to 150 mm. per minute (Staudacher-



*Dalle Aste, 1941*). Contractions begin at the gastric end of an explant and proceed toward the rectal end (*Bisceglie, 1932*). The further the temperature of the medium deviates in either direction from about 40° C., the more adversely is muscular activity affected (*Machii, 1930a; Bisceglie, 1932*).

The development of reactivity to drugs also coincides with the initial differentiation of the mesenchymal portion of the intestine. Barium chloride first exerts its stimulating effect on the (excised) gut on the chick's fifth day of incubation, and chinin, emetin, papaverin, and veratrin on the sixth day; the inhibitory action of the latter four muscle poisons is evinced on the seventh day. Sensitivity to nerve poisons develops slightly later: to physostigmine on the sixth day, and to acetylcholin, adrenalin, atropin, and pilocarpin on the seventh day. The response of the embryonic gut to all the above drugs is qualitatively much the same as that of the adult intestine, but excitability increases throughout incubation (*Machii, 1930b; Suma, 1931a, 1931b*). Among drugs that are stimulating in weak concentration, starting on the sixth day, and paralyzing in strong concentration, starting on the seventh day, are various alcohols (methyl, ethyl, propyl, butyl, and amyl); narcotics, such as chloral hydrate, amylene hydrate, sulfonal, and sodium veronal and luminal; acetic acid, acetone, diethyl ketone, and paraldehyde; and magnesium and ammonium chloride. As incubation proceeds, the intestine becomes more sensitive to the stimulating action of these substances and more resistant to their paralyzing effect (*Suma, 1931c, 1931d, 1931e, 1931f*).

The epithelium and mesenchyme of the 11-day chick embryo's intestine are both very susceptible to injury by heat, as compared with other embryonic tissues (skin, heart, amnion). Exposure of cultured intestinal fragments to a temperature of 48° C. for 15 to 30 minutes kills all freshly grown epithelial cells, although regeneration from the center of the culture can still take place. The mesenchyme is more resistant and can regenerate after being heated for 40 to 74 minutes (*Szarski, 1946*).

### *The Caeca*

The formation of folds begins in the caeca of the chick embryo on the tenth day of incubation, when a few irregular elevations appear. These grow higher more rapidly in some places than in others and become subdivided into smaller protuberances which, on the fourteenth day, may be regarded as low villi. The villi are of various sizes and are placed close together but show no definite arrangement (*Hilton, 1902*). In the distal part of the caecum, according to Pap (*1933*), the mesenchyme is passively thrown up into a few large folds by the invagination of epithelial gland anlagen which begin to form on the eleventh day when mitotic activity increases in the epithelium.



In the tufted penguin (*Eudyptes chrysolophus*), the long tubular glands that characterize the small caeca of the adult have not developed by the 23- to 25-day stage. At this time, the caeca are lined with a single layer of cylindrical epithelial cells and have no distinguishing features (Bartram, 1901).

### *The Large Intestine*

In the epithelium of the large intestine, mitotic activity begins to increase at the end of the chick's fourth incubation day, that is, somewhat earlier than in the lining of the small intestine. The wave of mitotic activity progresses caudocraniad. Approximately on the sixth day, three or four large epithelial folds project into the lumen so far that their apices meet and fuse, thus occluding the intestinal cavity (Minot, 1900a; Pap, 1933). At this time, the mesenchyme is in its initial stages of differentiation into muscular and submucosal layers (Minot, 1900a; Forssner, 1907).

Occlusion of the lumen has extended to the cranial end of the large intestine by the chick's eleventh day (Forssner, 1907). The epithelial tissue filling the cavity is never compact, since it contains many intercellular spaces (Minot, 1900a; Forssner, 1907; Pap, 1933) which tend to be oriented radially. By the time the upper end of the large intestine is closed, recanalization has begun at the lower end (Pap, 1933). A secondary lumen, lined with cylindrical epithelium, is established by the twelfth day (Minot, 1900a) through the degeneration of the central epithelial cells (Pap, 1933) which are cuboidal (Minot, 1900a).

Villi arise in the large intestine in the areas between the crypts of Lieberkühn, which, in the zebra parakeet (*Melopsittacus undulatus*), appear on the fourteenth day in greater abundance than in the small intestine (Joos, 1941). In the large intestine of the chick, villus formation begins on the eighteenth day and proceeds caudocraniad (Pap, 1933). The villi develop from small elevations of epithelium into each of which protrudes a connective tissue core (Hilton, 1902) containing an aggregation of red blood cells (Pap, 1933). Villi in this part of the intestine are lower, thinner, and less numerous than those in the small intestine.

On the fourteenth day of incubation, argentaffin cells are present in the large intestine of the chick (Simard and Campenhout, 1932), and mucin-secreting goblet cells appear in that of the zebra parakeet, *Melopsittacus undulatus* (Joos, 1941). Goblet cells are very numerous by the end of incubation (Joos, 1941).

The circular layer of muscle appears on the chick's seventh day and begins to expand on the tenth day. Its expansion probably stretches the intestinal wall and may thus be a factor in reopening the lumen (Pap, 1933). The longitudinal muscle layer is present on the eleventh day



(Minot, 1900a) but is only one third as thick as the circular layer at the end of incubation (Pap, 1933).

## THE CLOACA AND THE BURSA OF FABRICIUS

The alimentary tract of birds terminates in an enlarged segment known as the cloaca. The cloaca is said to consist of three anteroposteriorly successive chambers, but this division is indistinct in adults and hence rather artificial. The most cranial chamber, the coprodaeum, is a direct continuation of the large intestine; rarely, the intestine and the coprodaeum are separated by a low projecting fold. The second chamber, or urodaeum, is smaller than the coprodaeum and lies directly posterior to it. The urodaeum receives the urogenital ducts dorsolaterally; its cranial and caudal limits are indicated by fairly distinct folds. The last chamber, or proctodaeum, leads to the outside through the anus and is closed externally by a strong sphincter muscle. Unlike the remainder of the cloaca, the proctodaeum is of ectodermal origin. In young birds, and also in the adults of certain Ratitae, the bursa of Fabricius lies along the dorsal surface of the terminal intestine and its duct opens into the dorsal wall of the proctodaeum. The bursa, which is peculiar to birds, is a structure of unknown function and is derived from the endoderm of the embryonic cloaca.

### The Primitive Cloaca

The primitive cloaca becomes recognizable during the period when the rotation of the embryo's tail changes the positions of the allantoic and tail gut diverticula relative to each other. At this time, the cloaca is merely a high, narrow space, lined with endoderm. As the allantois rotates to a ventral position, the original ventrocaudal wall of the allantois becomes the floor of the cloaca. The cranial limit of the cloaca, corresponding to the cranial boundary of the allantois, is indefinite at first but becomes clearly indicated as the allantois begins to evaginate. The caudal boundary of the primitive cloaca may be placed temporarily at the level where the tail gut takes its origin.

### The Reduction of the Tail Gut

A process associated with the early development of the cloaca is the disappearance of the tail gut or caudal intestine. As previously mentioned, the tail gut begins to extend caudad into the tail about at the time when the allantois assumes its ventral position. The caudal intestine elongates with the tail for a short time and attains its greatest length in the chick, duck (*Anas platyrhynchos*), or lapwing (*Vanellus vanellus*) embryo at the stage of 40 to 44 somites (Boyden, 1922a; Hafferl, 1926), reached by the chick during the second half of the third incubation day. At this time,



the tail gut, of much smaller caliber than the cloaca, is patent and extends without interruption from the dorsocaudal corner of the cloaca to the blastema of the tail bud, which represents a condensation of the primitive node and the anterior part of the primitive streak. At the ventrocaudal border of the cloaca, behind the allantois, can be seen the thin cloacal membrane or anal plate, which lies at the depths of the somatic tail fold; this membrane is composed of fused ectoderm and endoderm and represents the remnant of the posterior part of the primitive streak (Gasser, 1880; Jolly, 1915; Gaertner, 1949).

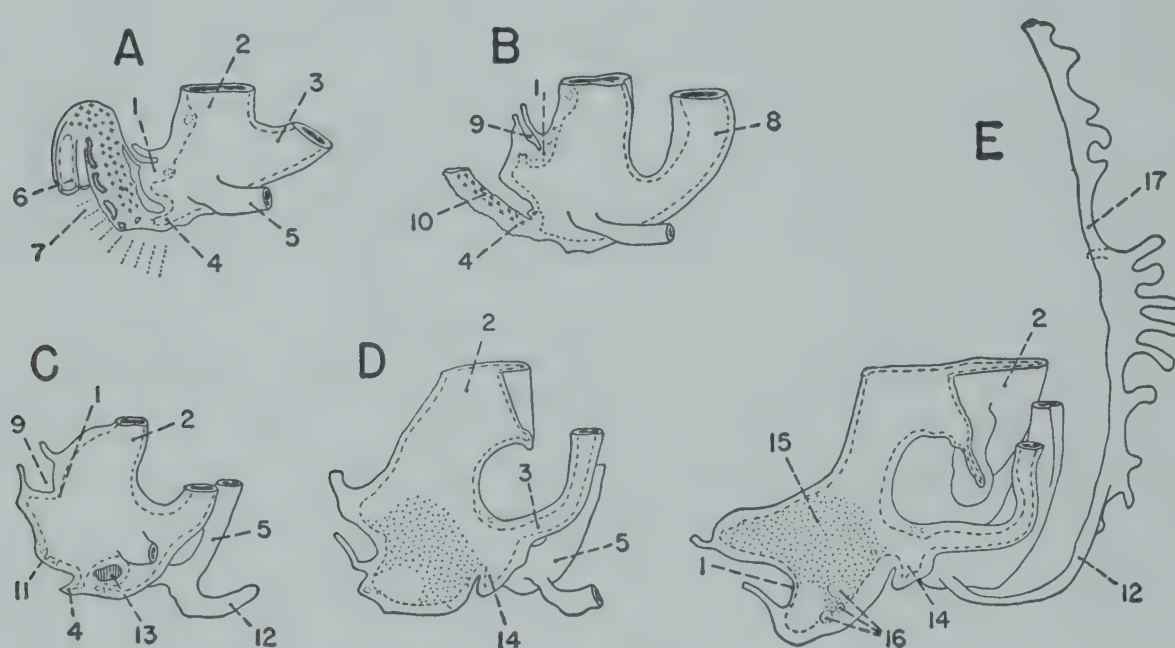


Fig. 197. Early development of the cloaca and adjacent structures in the ostrich (*Struthio camelus*) embryo shown by reconstructions of the cloacal region in progressively older ostrich embryos. (Redrawn with modifications after Boyden, 1922b.)

A, in the 56-somite ostrich embryo; B, in the 7-mm. embryo; C, in the 10.2-mm. embryo; D, in the 13.5-mm. embryo; E, in the 18-mm. embryo. All  $\times 20$ .

Cavities are outlined with broken lines; dotted lines indicate vacuoles and somites; dotted areas show regions where opposite walls of the cloaca are nearly in contact; crosses represent primitive streak remnants; and an area of disintegration is vertically lined.

1, anal plate; 2, allantois; 3, rectum; 4, caudal intestine; 5, Wolffian duct; 6, neural tube; 7, the fifty-sixth somite; 8, large intestine; 9, proctodaeum; 10, primitive streak vestige; 11, diverticulum a; 12, ureter; 13, area occupied by mesenchyma; 14, diverticulum c (urodaecal sinus); 15, urodaecal membrane; 16, primordia of cloacal bursa of Fabricius; 17, constriction of pelvis caused by umbilical artery.

The tail gut now begins to degenerate. In the tern (*Sterna hirundo*), according to Boyden (1922a), its destruction is accomplished much as it is in reptiles and mammals. First, the caudal intestine becomes attenuated and occluded at its proximal end, where it soon ruptures and is resorbed; occlusion and resorption then progress caudad until the entire tail gut has disappeared. Reduction of the caudal intestine in the chick (Keibel and Abraham, 1900), the lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909; Hafferl, 1926), and the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901), has been described as occurring in a similar way during



the period between the 37- and 49-somite stages. Boyden (1922a), however, found that the disintegration of the tail gut in the duck (*Anas platyrhynchos*) and the chick differed somewhat from the same process in the tern (*Sterna hirundo*). He observed that cellular degeneration begins at 41- to 45-somite stages at the junction of the tail gut with the cloaca and progresses craniad into the cloaca and caudad into the tail gut (Fig. 199-B<sub>1</sub> and B<sub>2</sub>). In the duck, the tail gut very soon becomes occluded at its proximal end; in the chick, however, the epithelium of the caudal intestine degenerates and is removed by phagocytosis too rapidly for occlu-

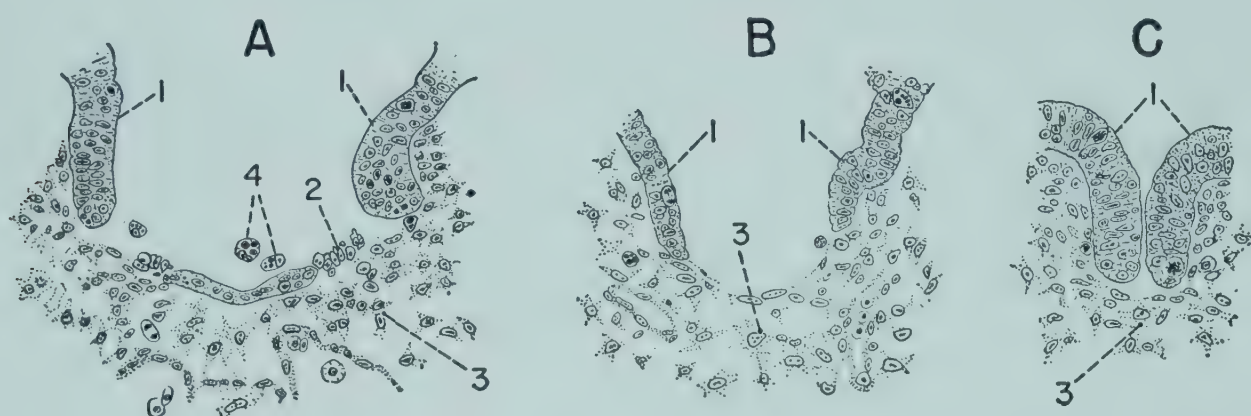


Fig. 198. Formation, maximum extent, and closure of the cloacal fenestra in the developing chick embryo, shown in histological sections. (Redrawn with modifications after Boyden, 1922a.)

A, obliquely transverse section through the cloaca of the chick at 2 days 20 hours, showing the cloacal fenestra as bilateral gaps in the cloacal wall; B, section through the cloacal fenestra at its maximum extent, in the embryo of 3 days 4 hours; C, section through the cloacal fenestra of the chick at 3 days 18 hours, showing the cloacal fenestra at the last stage before closure, reduced in size to a small slit between the two side walls which have approximated each other. All  $\times 150$ .

1, epithelial lining of cloaca; 2, degenerating epithelium; 3, mesenchyme; 4, phagocytes.

sion to occur. The disintegration of the ventral wall of the chick's tail gut appears to be an extension of a degenerative process originating in an undifferentiated mass of tissue located in the ventral curvature of the tail. Boyden (1922b) noted that a third method of reducing the caudal intestine is found in the ostrich (*Struthio camelus*). By the 56-somite stage, the continuous lumen of the tail gut has been broken up into isolated cavities by the irregular adhesion of the walls (Fig. 197-A). The remnants of the lumen then disappear, so that the tail gut is transformed into a solid strand (Fig. 197-B). The distal portion of this strand ruptures and is resorbed, leaving the most proximal part of the caudal intestine attached to the cloaca (Fig. 197-C). The stump of the tail gut then seems to be shifted craniad along the dorsal wall of the cloaca to a level opposite the allantois to become part of the urodaeum (Fig. 197-D and E).

Studies of the chicken, duck, *Anas platyrhynchos* (Boyden, 1922a), and lapwing, *Vanellus vanellus* (Hafferl, 1926), have shown that a portion of the dorsal wall of the cloaca is removed by the forward extension of the



same degenerative process that destroys the tail gut (Fig. 198-A; cf. Fig. 199-B<sub>2</sub>). In the chick embryo, the foramen formed in this way reaches its maximum size during the first half of the fourth day of incubation (Fig. 198-B) and is closed before the end of the same day (Fig. 198-C) by the approximation of the lateral walls of the cloaca (Boyden, 1922a).

### The Development of the Urodaeum and the Coprodaeum

Early in development, the primitive cloaca undergoes modifications that adapt it to receive the excretory products of the embryonic kidneys; it is thus transformed into the urodaeum. Observations of the chick (Keibel and Abraham, 1900), the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901), and the lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909), indicate that the blind ends of the Wolffian ducts reach the level of the cloaca about at the time the tail gut begins to degenerate, or slightly earlier (Fig. 199-A). Several dorsolateral diverticula now form in the wall of the cloaca on either side, opposite the distal portion of each Wolffian duct. Late in the chick's third day of incubation (Duval, 1889; Boyden, 1922a), the medial wall of the duct fuses with several of the diverticula along a roughly horizontal line extending from the caudal margin of the cloaca to the level of the allantois. Rupture of the fused tissues then gives the Wolffian duct an elongated opening into the cavity of the cloaca (cf. Fig. 199-B<sub>1</sub> and B<sub>2</sub>). By the end of the fourth day, the mouth of the Wolffian duct is a smaller, round outlet corresponding in position to the most cranial part of the original opening (Boyden, 1922a).

The lumen of the cloaca now begins to enlarge transversely at the level where the Wolffian ducts enter, so that a cross section of this region has the shape of an inverted triangle (Gasser, 1880; Pomayer, 1902). The lower end of each Wolffian duct also enlarges between the cloaca and the mouth of the ureter, and the dilated segment gradually merges indistinguishably with the cloaca (Fig. 199-C<sub>1</sub>, C<sub>2</sub>, D, and E). Also, the cranial wall of the cloaca becomes more deeply indented between the allantois and the hind-gut, some of the cloaca thus being taken up into the allantoic stalk and the hind-gut (Hafferl, 1926). As a result, the mouths of the Wolffian ducts seem to be shifted craniad (cf. Fig. 199-D and E). Meantime, the lateral walls of the cloaca have begun to fuse together just in front of the anal plate, obliterating the caudal end of the cloacal lumen and initiating the formation of a lamella in the sagittal plane (Fig. 200-A and B); this lamella, which is continuous with the anal plate, is the urodaeal membrane (ural or cloacal plate). The cloacal cavity is further reduced in size as the urodaeal membrane extends forward. In the 5- to 6-day chick (Boyden, 1922a), as in the 12-mm. lapwing, *Vanellus vanellus* (Hafferl, 1926), and the 15-mm. duck, *Anas platyrhynchos* (Pomayer, 1902), the lumen of the cloaca lies between the urodaeal membrane and the occluded hind-gut and



is therefore a more or less vertical channel connecting the Wolffian ducts with the allantois (Fig. 200-B). The primitive cloaca has thus become the urodacum. Its lumen, or urodacal sinus (Boyden, 1922a), is T-shaped in cross section, the dorsolateral expansions of the cloacal cavity having been

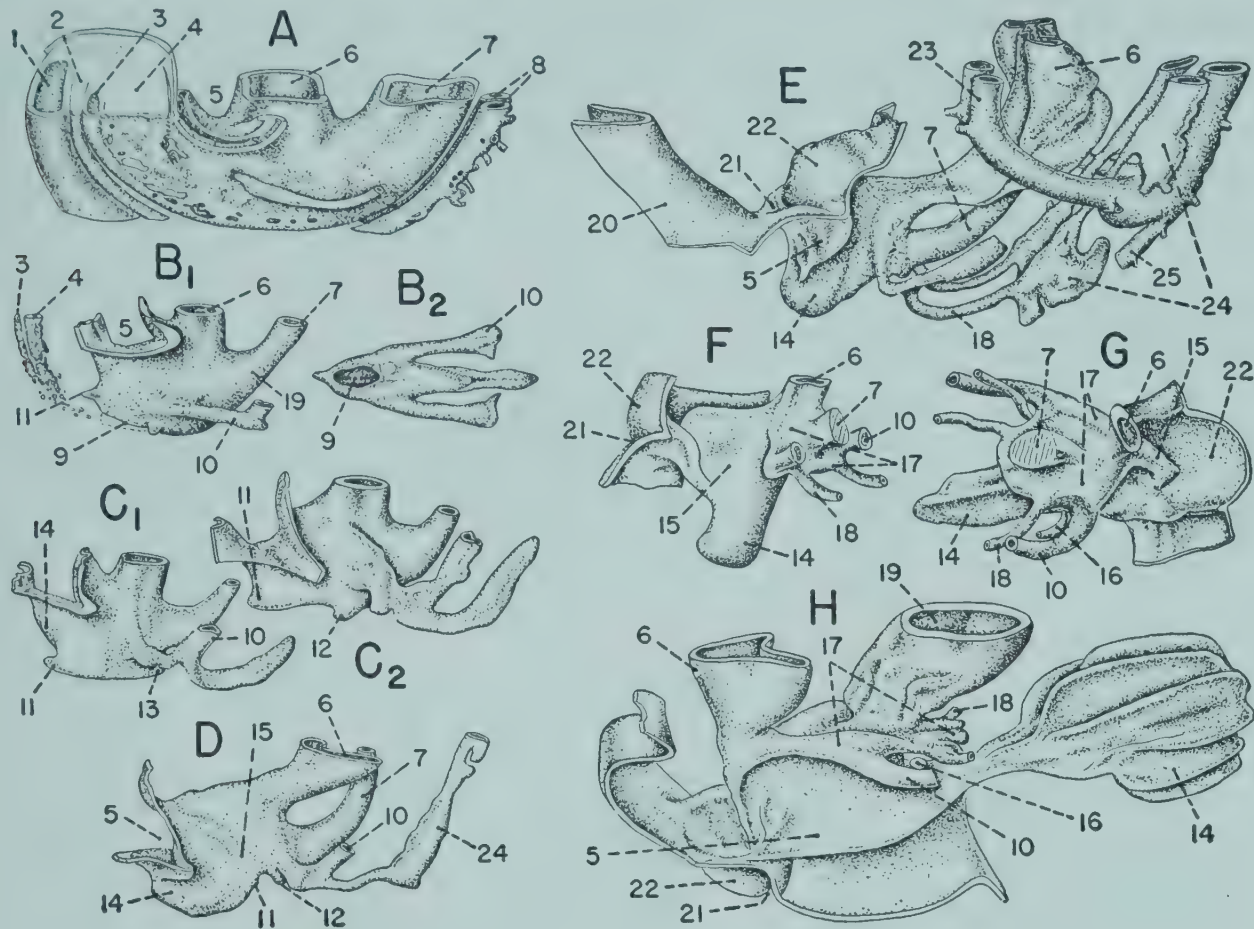


Fig. 199. Development of the cloaca in the chick embryo, shown by models. (Redrawn with modifications after Boyden, 1922a, 1924.)

A, at 2 days 18 hours, showing the initial steps in the formation of the cloacal fenestra by disintegration of the cloacal wall; B<sub>1</sub> and B<sub>2</sub>, at 3 days 6 hours, showing the cloacal fenestra at its maximum extent as it appears in a lateral view, B<sub>1</sub>, and from below, B<sub>2</sub>; C<sub>1</sub> and C<sub>2</sub>, at 4 days 3 hours, when diverticula *a*, *b*, and *c* have developed; D, at 5 days, showing a well-developed bursa of Fabricius; E, at 5 days 15 hours, showing lateral invaginations of the proctodaeum meeting the bursa of Fabricius; F, at 6 days 7 hours, showing the differentiated urodacal sinus; G, at 8 days 1 hour, when rectum is occluded; H, at 11 days, with bursa differentiated into stalk and plicated gland, and cloaca divided into the three transverse parts characteristic of the adult; proctodaeum (ectodermal origin); urodacum (cloaca proper receiving urogenital ducts); and the coprodacum (rectal ampulla with its "zona columnaris"). All  $\times 20$ .

1, medullary tube; 2, notochord; 3, caudal intestine; 4, primitive streak mass; 5, proctodaeum; 6, allantois; 7, rectum; 8, dorsal aortae; 9, cloacal fenestra; 10, Wolffian duct; 11, diverticulum *a*; 12, diverticulum *b*; 13, diverticulum *c*; 14, bursa of Fabricius; 15, urodacal membrane; 16, Müllerian duct; 17, urodacum or urodacal sinus; 18, ureter; 19, coprodacum; 20, cauda; 21, anus; 22, phallus; 23, umbilical artery; 24, metanephric pelvis; 25, caudal artery.

greatly increased in size through the continued addition of the distal portions of the Wolffian ducts (Hafferl, 1926). In the 8-day chick, (Boyden, 1922a) or 20-mm. lapwing, *Vanellus vanellus* (Hafferl, 1926), the antero-posterior diameter of the urodacal sinus has been reduced to a mere fissure



(Fig. 200-C and D). The transverse diameter is even greater than before (Fig. 199-G), because the Wolffian ducts have been absorbed into the urodaeum as far as the ureters. The latter have rotated from their original position dorsal to the Wolffian ducts and now enter the urodaeum medial to the latter (cf. Fig. 199-G). The ureters are received into a dorsal prolongation of the urodaeal sinus, first apparent in the 4-day chick (cf. Fig. 199-C<sub>1</sub> and C<sub>2</sub>) as a median diverticulum at the junction of the hind-gut

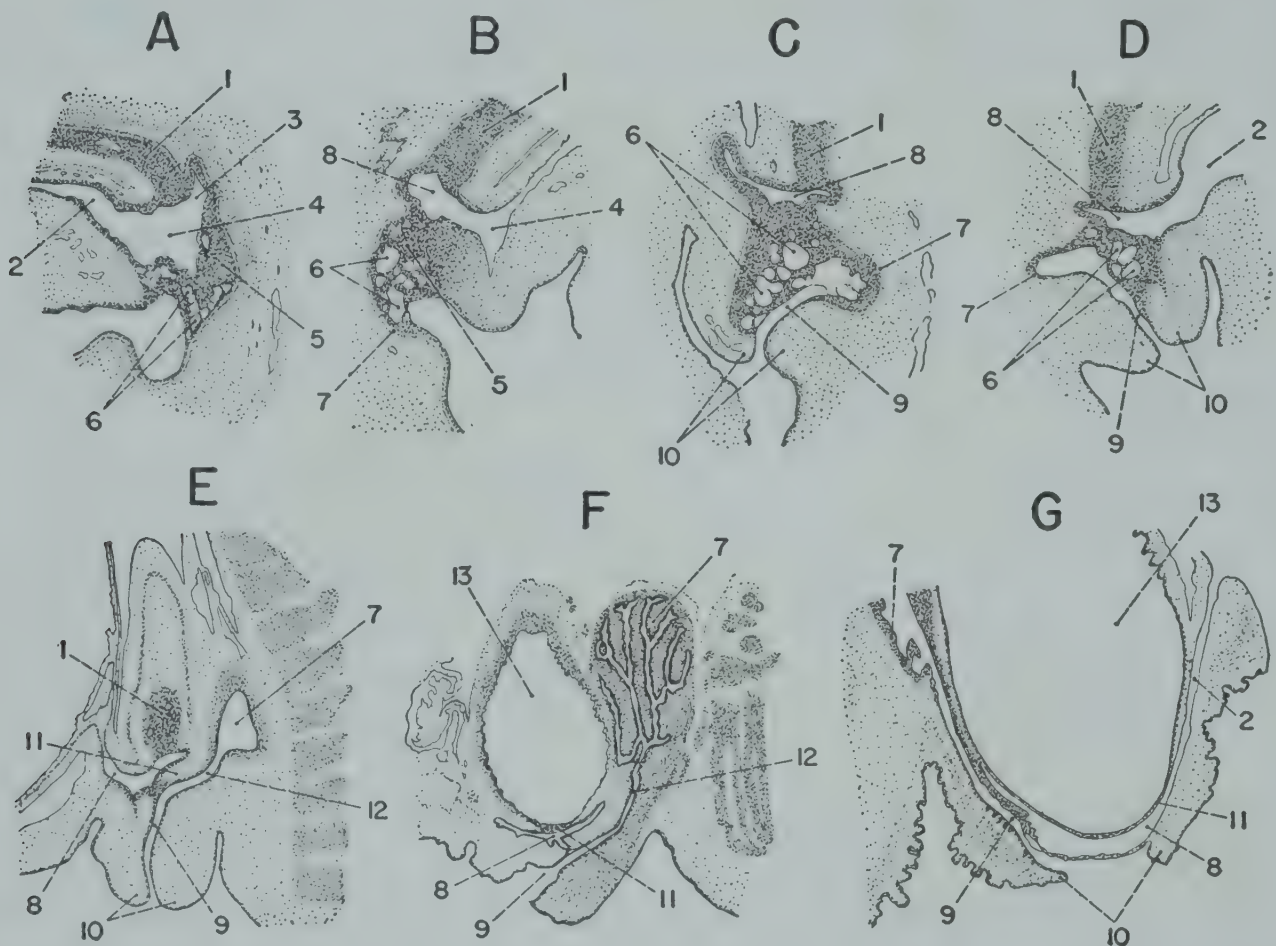


Fig. 200. Stages in the development of the cloaca and the bursa of Fabricius in the chick embryo, as shown by median sagittal sections. (Redrawn with modifications after Jolly, 1915.)

A, from a 5-day embryo ( $\times 20$ ); B, from a 6-day embryo ( $\times 20$ ); C, from a 7-day embryo ( $\times 20$ ); D, from an 8-day embryo ( $\times 20$ ); E, from a 9-day embryo ( $\times 6$ ); F, from a 13-day embryo ( $\times 6$ ); G, from an 18-day embryo ( $\times 6$ ).

1, large intestine; 2, allantoic duct; 3, Wolffian (urogenital) duct; 4, cloaca; 5, epithelial mass in cloaca; 6, vacuoles in epithelial mass; 7, bursa of Fabricius; 8, urodaeum; 9, proctodaeum; 10, anal papilla; 11, urodaeal membrane; 12, duct of bursa of Fabricius; 13, coprodaeum.

and the cloaca (Boyden, 1922a). In the ostrich, *Struthio camelus* (Fig. 197-C, D, and E), it seems that this diverticulum consists of the cranial remnant of the caudal intestine (Boyden, 1922b).

The later development of the urodaeum is influenced by the differentiation of the coprodaeum. The coprodaeum is derived from the terminal segment of the hind-gut, although it is possible that at least the caudal part of the coprodaeum represents the original cranial portion of the primitive cloaca, added to the hind-gut (Hafferl, 1926). The formation of the



coprodaeum is initiated after the chick's eighth day of incubation (Boydén, 1922a), when the hind-gut, still occluded, is surrounded by undifferentiated mesoderm (Retterer, 1885). Gradually, the hind-gut becomes narrower at its junction with the urodaeum and wider immediately craniad to this level (Fig. 200-E). The narrowed portion, encircled by a ring of mesoderm (Gasser, 1880), is soon reduced to a thin epithelial stalk attaching the adjoining wider portion to the urodaeum. By the chick's tenth or eleventh day (Retterer, 1885) and the duck's (*Anas platyrhynchos*) thirteenth or fourteenth (Jolly, 1915), a lumen has begun to reappear in the widened segment of gut, and mucosal folds, 0.02 mm. high and covered with epithelium 0.04 mm. thick, are projecting into the cavity (Fig. 199-F); also, the outer muscular layers are distinguishable and the serosa has differentiated (Retterer, 1885). The lumen continues to dilate, and a large chamber, recognizable as the coprodaeum, is established within a day or two. As the coprodaeum expands, the mesodermal ring surrounding its stalk shows a corresponding increase in diameter, while simultaneously decreasing in thickness.

In the 13-day chick embryo or the 18-day duck (*Anas platyrhynchos*) embryo (Jolly, 1915), the coprodaeum is so large that its convex caudal wall bulges into the urodaeal cavity (Fig. 200-F), and the urodaeum is thus transformed into a shallow, cup-shaped vesicle clasping the caudal end of the coprodaeum (Pomayer, 1902). The folds within the coprodaeum are now 0.06 mm. high, but the muscularis mucosae is not yet distinguishable (Retterer, 1885). Between the coprodaeum and the urodaeum there is a thin membrane formed of three layers—the coprodaeal epithelium, the urodaeal epithelium, and an intervening layer of connective tissue (cf. Fig. 200-F); in the center of this membrane is an epithelial plug representing the remnant of the solid stalk of the hind-gut (Pomayer, 1902; Jolly, 1915). During the last days of incubation, the membrane grows extremely thin (Fig. 200-G), but it persists almost to the hour of hatching before it ruptures. Its site is marked by a ridge on the internal surface of the cloaca (Pomayer, 1902). Cranially to this ridge, at the level of the coprodaeum, the cloaca is lined with a single layer of prismatic epithelial cells which extends uniformly over the villi and the spaces between them; no crypts develop in this part of the intestinal tract. Caudally to the ridge, the urodaeal portion of the cloaca is lined with a similar epithelium covered by a layer of squamous cells (Retterer, 1885).

The urodaeum is separated from the proctodaeum as well as the coprodaeum until close to hatching. Before the proctodaeum differentiates, the anal plate is extended craniad to form the urodaeal membrane, as mentioned previously. This membrane is a thin, vacuolated plate of epithelium standing vertically in the midline at the rear of the urodaeal sinus (cf. Fig. 200-B). At first, the lateral walls of the urodaeal sinus merge directly into



the urodaeal membrane. As the urodaeal sinus expands transversely and flattens anteroposteriorly, it acquires a true caudal wall (cf. Fig. 200-D), from whose midline the urodaeal membrane extends caudad, proliferating along the cranial border of the proctodaeum (Gasser, 1880; Hafferl, 1926). With the development of the proctodaeum and the bursa of Fabricius behind the urodaeum, a thick zone of mesoderm comes to intervene between these structures and the caudal wall of the urodaeum—except, of course, in the midline, which is still occupied by the urodaeal membrane. During the period when the coprodaeum is beginning to differentiate, the urodaeal membrane is gradually reduced in dorsoventral extent and is transformed into an epithelial strand passing through a thick mesodermal wall which stands between the urodaeum and the proctodaeum (cf. Fig. 200-E). As the urodaeum is progressively deformed by the caudad protrusion of the coprodaeum, the caudal urodaeal wall bulges outward, following the curvature of the cranial wall (cf. Fig. 200-F), and grows thinner. In the 18-day chick embryo or the 22-day duck (*Anas platyrhynchos*) embryo, the caudal wall of the urodaeum is close to its cranial wall and consists of a thin diaphragm (cf. Fig. 200-G), in the center of which is the epithelial remnant of the urodaeal membrane; the remainder of the diaphragm is composed of connective and vascular tissue lying between two epithelial layers. The epithelium on one side of the diaphragm—said to be both the urodaeal (Jolly, 1915) and the proctodaeal (Retterer, 1885) side—now sends out buds which grow through the connective tissue and join the epithelium on the other side. These solid epithelial strands, which are especially numerous around the periphery of the diaphragm, vacuolize and are replaced by fissures; the rudiment of the urodaeal membrane shares the same fate (Jolly, 1915). According to Gasser (1880), the urodaeum communicates with the proctodaeum on the chick's seventeenth day, and the opening is surrounded by folds which are covered with epithelium. Jolly (1915), however, stated that the entire diaphragm finally disappears, but not until the last day of incubation.

### The Development of the Proctodaeum and the Bursa of Fabricius

The cavity of the proctodaeum is lined with ectoderm and does not become confluent with the cavity of the endodermal cloaca until the incubation period is almost completed. During its development, the proctodaeum is closely associated with the bursa of Fabricius, which is derived from endodermal tissue (Bornhaupt, 1867) proliferated at the dorsocaudal margin of the urodaeal membrane.

The approximate site of the anal opening is indicated early in incubation when the tail fold forms opposite the anal plate or cloacal membrane. The anal plate, situated at the ventrocaudal corner of the primitive cloaca when the latter first becomes recognizable, has rotated to a ventral position



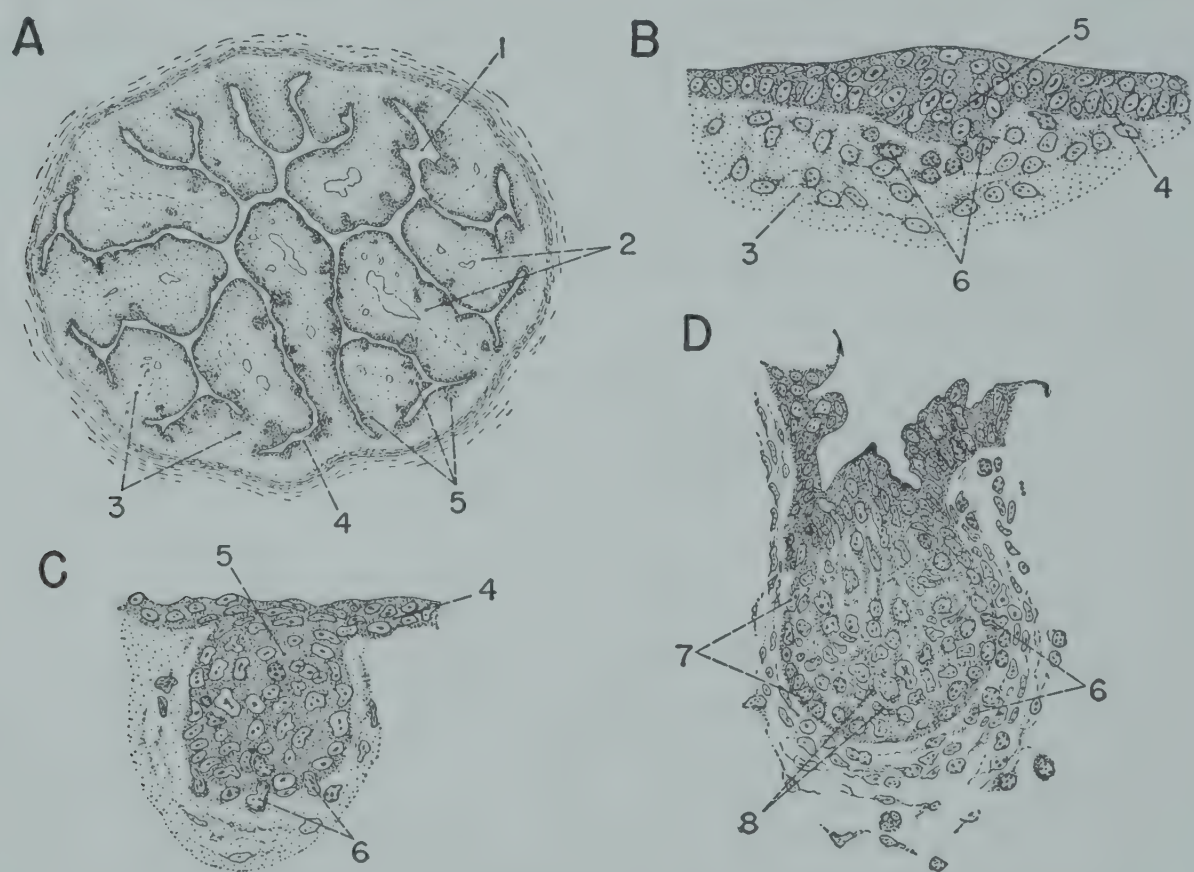
by the time the caudal intestine begins to degenerate. During the chick embryo's fifth day of incubation (*Rathke*, 1825, *p.* 70; *Gasser*, 1880; *Jolly*, 1915), and on the fifth and seventh days in different species of ducks, *Anas platyrhynchos* (*Pomayer*, 1902; *Jolly*, 1915), two protuberances appear in succession on the ventral body surface, the first one anterior to the anal plate and the second one posterior to it (cf. Fig. 199-C<sub>1</sub>, C<sub>2</sub>, and D; Fig. 200-B). These are the anterior and posterior anal lips; they become confluent at the sides to form the circular anal papilla or genital tubercle. The elevation of the anal lips produces between them a groove lined with ectoderm that was previously on the body surface. This groove is the primitive proctodaeum (cf. Fig. 199-D). At its mouth, the proctodaeum is an orocaudally compressed slit transverse to the long axis of the body (*Goette*, 1867, *p.* 31), but it grows smaller and more nearly round near its blind end. As the proctodaeum deepens with the growth of the anal papilla, its fundus becomes somewhat wider from side to side. In the 12.5 mm. goose (*Anser anser*) embryo, the transverse diameter of the proctodaeum is 0.8 mm. at the mouth and 0.28 mm. near the bottom, and the anteroposterior diameter is 0.25 mm. throughout; in the 17.8 mm. duck (*Anas platyrhynchos*), these dimensions are 0.84, 0.6, and 0.5 mm., respectively (*Pomayer*, 1902).

Meantime, the primordium of the bursa of Fabricius has appeared in the form of a median lamina of endodermal epithelium proliferated dorsad and caudad from the anal plate (*Minot*, 1900a), between the latter and the remnant of the caudal intestine (*Boyden*, 1922a). It is first seen about at the time that the anterior anal lip starts to protrude, or even earlier (cf. Fig. 199; Fig. 200). The bursal proliferation is in continuity cranially with the developing urodaeal membrane and extends above and behind the fundus of the proctodaeum in the median plane, the anal plate now being situated along the oral proctodaeal wall (*Gasser*, 1874; *Pomayer*, 1902). This developmental stage is seen in the 11-mm. pigeon (*Columba livia*) embryo (*Jolly*, 1915).

In the 6-day chick, *Gallus gallus* (cf. Fig. 199-E; Fig. 200-B), 7-day duck (*Anas platyrhynchos*), or 12-mm. pigeon (*Columba livia*) embryo (*Jolly*, 1915), the epithelial anlage of the bursa projects from the dorso-caudal corner of the urodaeal membrane as a slightly rounded protuberance with a convex caudal margin (*Minot*, 1900a; *Boyden*, 1922a). Like the urodaeal membrane itself, it contains numerous lacunae (*Wenckebach*, 1888) which apparently form as the result of cell liquefaction caused by an originally intracellular process of vacuolization (*Jolly*, 1915). In chick and duck embryos 24 hours older (*Boyden*, 1922a; *Jolly*, 1915), as in the 8-day goose (*Anser anser*) embryo (*Pomayer*, 1902), the bursal primordium bulges laterally and extends farther caudalward. The proctodaeum is now curved, with its blind dorsal end turned caudad (*Pomayer*, 1902; *Jolly*, 1915); its



caudal wall continues without a break into the ventrocaudal wall of the bursal outgrowth (cf. Fig. 200-C). The lacunae within the bursa are beginning to coalesce to form a lumen (*Wenckebach, 1888*) lying directly in line with the proctodaeal sinus. The two cavities remain separated for a short time by a small amount of endodermal tissue to which is closely applied the ectoderm of the proctodaeal fundus. In the chick, this epithelial barrier ruptures (cf. Fig. 200-D) on the seventh or eighth day (*Wenckebach, 1888; Boyden, 1922a; Jolly, 1915*). The bursa then rapidly rotates forward around the transverse body axis (*Bornhaupt, 1867*) so that



**Fig. 201.** The histological development of the bursa of Fabricius in the chick embryo. (Redrawn with modifications after *Jolly, 1915*.)

A, cross section through the bursa of Fabricius of a 14-day embryo ( $\times 40$ ); B, C, and D, sections of epithelial buds at 12, 14, and 18 days, respectively ( $\times 350$ ).

1, cavity of the bursa; 2, folds projecting into the cavity; 3, mesenchyme; 4, epithelium; 5, epithelial buds; 6, lymphoid cells of mesenchyme; 7, incipient epithelial border of early follicle; 8, degenerating epithelial cells.

it is oriented vertically (cf. Fig. 200-E) on the ninth day (*Jolly, 1915*) and lies almost parallel with the coprodaeum by the eleventh day (*Boyden, 1922a*). As it rotates, the bursa changes from round to oval in shape (*Bornhaupt, 1867*) and grows out on a cylindrical stalk whose long axis forms a slight angle with that of the proctodaeum (cf. Fig. 200-E). In the pigeon (*Columba livia*) and in ducks (*Anas platyrhynchos*), the bursa begins to alter its position before the confluence of the bursal and proctodaeal lumina, which first communicate with each other at the 14-mm. stage in the pigeon and on the eleventh or thirteenth day in ducks (*Pomayer,*



1902; Jolly, 1915). The passage formed by the union of proctodacum and bursal stalk is separated from the remainder of the cloaca until close to the time of hatching, when the caudal wall of the urodaeum ruptures in the manner previously described.

Soon after the bursal lumen is completely formed, longitudinal plicae begin to project into it (cf. Fig. 199-H). In the chick, the first plicae appear on the tenth (Jolly, 1915) or eleventh day (Bornhaupt, 1867; Retterer, 1885); by the fourteenth or fifteenth day, six or seven of the eleven or twelve plicae have grown far out into the lumen (Boyden, 1922a), whose cross section is thus transformed into several irregularly branched and radiating fissures (Fig. 201-A). Near the stalk of the bursa, the plicae are broad at the base and 0.1 to 0.25 mm. high, but as they go toward the fundus, they increase to a height of 1.0 mm. and their bases become narrower than their free ends (Retterer, 1885). In the duck (*Anas platyrhynchos*), only two plicae develop; these are on the ventral wall (Pomayer, 1902) and appear on the twelfth day (Jolly, 1915).

The bursa continues to grow until the end of incubation. In the chick, its length increases from 1.25 mm. on the eleventh day to 8 mm. on the twenty-first (Retterer, 1885).

#### *Histological Development of the Bursa of Fabricius*

The wall of the definitive bursa of Fabricius is composed, from periphery to lumen, of the following layers: a thin serosa; a muscularis, consisting either of circularly disposed fibers (Jolly, 1915) or of an outer layer of longitudinal fibers and an inner layer of circular fibers (Retterer, 1885); a mucosa; and an epithelial lining of cylindrical or cuboidal cells arranged in several rows. The mucosa is the thickest portion of the wall. Its connective tissue extends into all the plicae and forms a framework for the so-called follicles, which are embedded in it. The follicles are each made up of a central medulla and an outer cortex, except in certain birds (including the ostrich, *Struthio camelus*, rhea, *Rhea americana*, and various birds of prey) where the medulla partially or wholly surrounds the cortex. The medulla is in continuity with the lining of the epithelium. The cortex is denser and more darkly staining than the medulla and is separated from it by a limiting membrane which is continuous with the basal membrane beneath the epithelium. Both cortex and medulla contain a reticulum of anastomosed stellate cells whose meshes are filled with lymphoid cells; in the cortex, the reticulum is continuous with the surrounding fibrillar connective tissue.

About at the time that the lumen of the bursa appears, or shortly afterward, the initial differentiation of the mesodermal portion of the organ is indicated by the concentric arrangement of the mesenchymal cells sur-



rounding the epithelium (*Stieda*, 1880). The mesodermal capsule is thinned somewhat by the rapid early enlargement of the bursal lumen (*Bornhaupt*, 1867) but soon grows thick again. Hypertrophy of the mesodermal tissue then produces the first longitudinal plicae. The serosa, muscularis, and mucosa now begin to differentiate. In the 13-day chick embryo (*Retterer*, 1885), the serosa is 0.036 to 0.04 mm. thick; it is continuous with the abdominal peritoneum and, ventrally, with the mesorectum. The ventral surface of the bursa is applied against the dorsal surface of the coprodaeum (cf. Fig. 200-F), and the bursal serosa is fused with the serosa of the coprodaeum to form a sheet of tissue 0.12 to 0.15 mm. thick. In the muscular tunic, which is 0.024 to 0.03 mm. thick, the nuclei of the myoblasts are elongated but the cytoplasm is little differentiated. The mucosal layer is only 0.04 mm. thick between the plicae and is composed of fusiform cells. The plicae and the intervals between them are covered by three or four rows of epithelial cells forming a layer 0.018 to 0.03 mm. thick. Buds of thickened epithelium project into the mucosal layer.

These buds, which are the anlagen of the bursal follicles, begin to appear on the twelfth day in the chick (*Wenckebach*, 1888; *Jolly*, 1915) and on the eighteenth in the duck, *Anas platyrhynchos* (*Jolly*, 1915). At first, they are more or less conical, with broad bases (Fig. 201-B), but as they grow they become spherical (Fig. 201-C) and slightly pedunculated (*Bornhaupt*, 1867; *Stieda*, 1880; *Retterer*, 1885; *Jolly*, 1915). The adjacent mesenchymal cells arrange themselves concentrically around the buds (*Retterer*, 1885). Even before the budding of the epithelium begins, large amoeboid cells differentiate from some of the stellate mesenchymal cells and accumulate beneath the epithelium at the points where the buds will appear (cf. Fig. 201-B). As the buds form and grow, the amoeboid cells penetrate into them (cf. Fig. 201-C), insinuating themselves between the epithelial cells (*Jolly*, 1915). Many of the epithelial cells then undergo cytoplasmic vacuolization and nuclear degeneration (*Schumacher*, 1903; *Jolly*, 1915). Some of the persisting epithelial cells align themselves in a single row around the periphery of the bud (Fig. 201-D), immediately within the basal membrane (*Jolly*, 1915). Others are transformed into stellate cells which anastomose to form the reticular framework of the medulla (*Wenckebach*, 1888; *Retterer and Lelièvre*, 1910; *Jolly*, 1915). Although various investigators concluded that the epithelial cells also give rise to the lymphocytes enmeshed in this reticulum (*Retterer*, 1885; *Wenckebach*, 1888; *Schumacher*, 1903; *Retterer and Lelièvre*, 1910), the work of *Jolly* (1915) makes it appear more likely that the lymphocytes are derived from the invading mesenchymal cells. In the chick, the invasion of these cells is very marked from the fourteenth to the eighteenth day, after which time the number of lymphocytes is augmented by mitosis rather than by migration.



## THE LIVER AND THE GALL BLADDER

In most birds, the liver consists of a left lobe, situated ventral to the proventriculus and gizzard, and a somewhat larger right lobe, lying ventral to the pancreas, the open end of the duodenal loop, and part of the ileum. In some species, there is a third, intermediate lobe of small size. Bile secreted by the liver is carried to the duodenum by biliary ducts, usually two in number. One duct, known as the hepatic or hepatoenteric duct, arises in the left lobe. The other duct, arising in the right lobe, has the gall bladder inserted upon its course in the majority of species and hence is subdivided into a hepatocystic and a cystoenteric or cystic duct. In all or many members of a number of avian families, notably the Psittacidae, Cuculidae, and Columbidae, the gall bladder is lacking. The biliary ducts join the duodenum at levels that vary greatly from one species to another, but the mouths of the ducts are most often found near the distal end of the ascending limb of the duodenal loop.

In its finer structure, the avian liver resembles the mammalian, although its parenchyma is not arranged in lobules. The hepatic cells are aligned in double or single rows (depending upon the species) to form what have variously been described as trabeculae, tubules, or plates. These are anastomosed into a vast, complex network intermeshed with a similarly ramified vascular network. Between every single or double row of cells there is, on one side, an extremely narrow bile canaliculus continuous with the biliary ducts, and, on the other side, a vascular space of capillary size, known as a sinusoid. The endothelial and fixed macrophage cells lining the sinusoids are in direct contact with the parenchyma.

The liver is both an exocrine and an endocrine gland, although its parenchymal cells are all of a single type. It performs the same diversified and complex functions in birds as in mammals. Among these functions may be mentioned the secretion of bile, the metabolism and storage of carbohydrates, fats, proteins, and vitamins, and the formation of urea, fibrinogen, and heparin.

The liver and the gall bladder and their respective ducts are all derived from the same endodermal primordium. Although some details of liver development vary greatly among birds of different species and even among individuals of the same species (*Hildebrandt, 1902; Elias, 1955*), its general course is the same. The primary hepatic anlage forms on the cranial wall of the anterior intestinal portal and promptly becomes divided distally into two secondary primordia, which grow forward in close association with the ductus venosus. Their proximal portions differentiate to form the gall bladder and the biliary ducts, and their distal portions give rise to the liver by proliferating tissue in an intimate relationship with endothelial tissue proliferated from various blood vessels.



### The Formation of the Primary and Secondary Hepatic Primordia

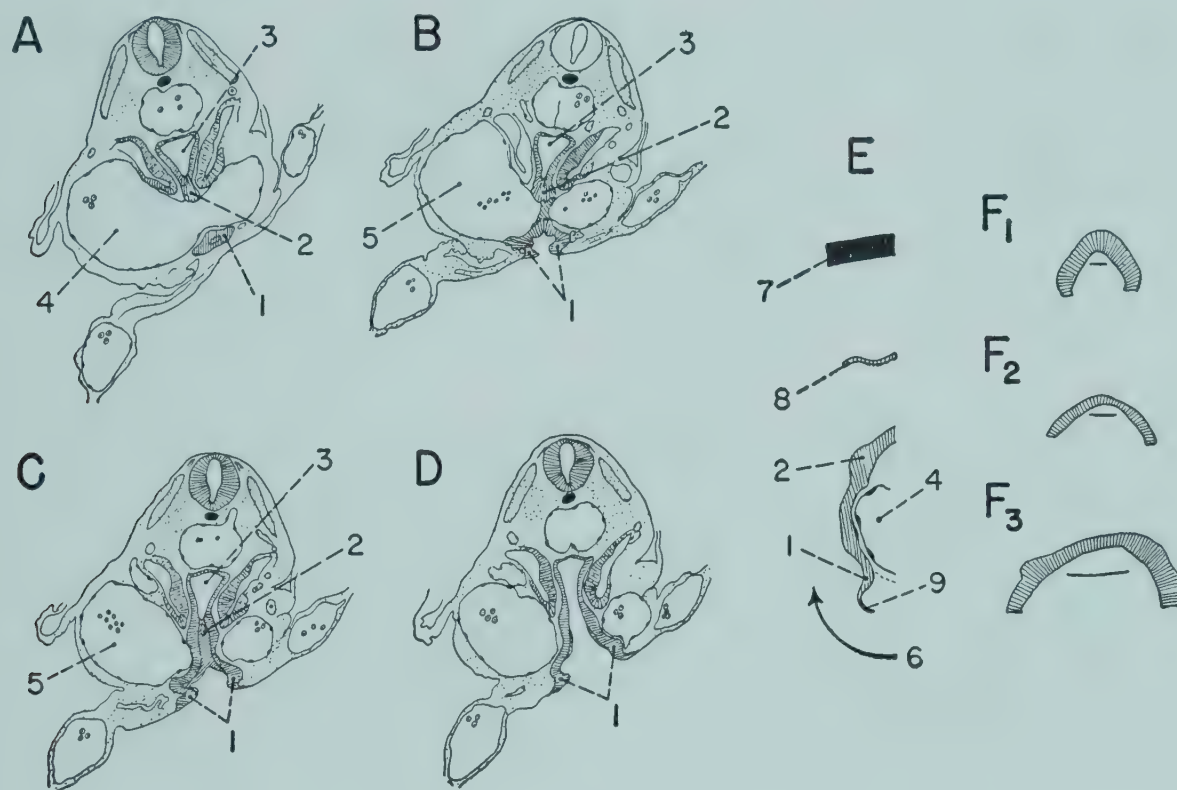
At the head-process stage, when the capacity of the mesendoderm to form liver in grafts begins to surpass that of the mesectoderm (*Hunt, 1937a*), liver potency is concentrated in two bilateral areas, one on each side of the head-process (*Rudnick, 1932; Rawles, 1936*). At this time and in early somite stages, liver potency occurs in close association with heart potency (*Willier and Rawles, 1931a, 1935; Dalton, 1935; Kumé, 1935; Rawles, 1936; Hunt, 1937a*). When the bilateral areas of prospective liver cells are brought together in the midline in the course of fore-gut closure, they fuse to form a single median primordium. The original bilaterality of the liver rudiment is often apparent (*Keibel, 1901; Hildebrandt, 1902*) and accounts for much of the variability in the structure of the early liver anlage.

The first visible liver primordium is seen in the chick close to the end of the second day of incubation, when there may be 16 to 23 somites (*Keibel and Abraham, 1900; Kingsbury, Alexanderson, and Kornstein, 1956*). Liver development begins at comparable stages of 20 to 23 somites in the zebra parakeet, *Melopsittacus undulatus*, and the European lapwing, *Vanellus vanellus* (*Abraham, 1901; Grosser and Tandler, 1909*). Typically, the primary hepatic anlage of the chick is an endodermal evagination extending vertically or obliquely across the angle formed by the reflection of the fore-gut into the yolk sac; that is, it is situated wholly or partially on the cranial wall of the intestinal umbilicus (*Brouha, 1898b; Choronshtitzky, 1900*). Usually, it is narrow dorsally and wide ventrally (*Brouha, 1898a; Hildebrandt, 1902*). It is demarcated from the gut and the yolk sac by slight constrictions, one at either end (*Brouha, 1898a, 1898b*). The portion between its dorsal and ventral extremities fits into the angle made by the two lateral vitelline omphalomesenteric veins as they join immediately cranial to the anterior intestinal portal to form the sinus venosus. A similar hollow diverticulum (Fig. 202) has been observed in the duck, *Anas platyrhynchos* (*Hildebrandt, 1902; Weber, 1902b*), and the scops owl, *Otus scops* (*Hildebrandt, 1902*). A solid cellular proliferation, however, has been found in some birds, including the gull, *Larus* sp. (*Hammar, 1893*); the zebra parakeet, *Melopsittacus undulatus* (*Abraham, 1901*); the Australian cockateel, *Nymphicus hollandicus* (*Hildebrandt, 1902*); the pigeon, *Columba livia* (*Brouha, 1898b; Hildebrandt, 1902*); the red-winged blackbird (*Agelaius phoeniceus*); and the sparrow, *Passer domesticus* (*Elias, 1955*). The solid anlage soon becomes hollow; in the sparrow, it contains a lumen by the end of the third day of incubation (*Elias, 1955*).

The secondary liver primordia appear in the form of diverticula or buds, depending upon whether the primary anlage is evaginated or proliferated. They can be identified a few hours after the primary rudiment appears and



are merely craniad prolongations of its dorsal and ventral extremities, the dorsal of which is often situated somewhat more cranially. They probably form because the caudal border of the ductus venosus prevents the primary rudiment from growing forward undivided (*Brouha, 1898b; Choronshtzky, 1900*). The dorsal or cranial liver primordium is dorsal to the sinus venosus or the ductus venosus, and the caudal primordium is ventral to it (*Shore, 1891; Felix, 1892; Hammar, 1893, 1897a; Hildebrandt, 1902*) (cf. Fig. 204-A). The cranial primordium appears very soon after or sometimes



**Fig. 202.** A very early stage of liver formation in the duck (*Anas platyrhynchos*), shown by sections of liver primordium in a 25-somite embryo. (Redrawn with modifications after Hildebrandt, 1902.)

A, B, C, and D, cross sections of the liver primordium at successively more caudal levels ( $\times 25$ ); E, schematic profile reconstruction of the liver primordium ( $\times 55$ );  $F_1$ ,  $F_2$ , and  $F_3$ , schematic representations of frontal sections through the cranial, middle, and caudal portions of the liver primordium, respectively; the horizontal line indicates the level of the section shown in D ( $\times 55$ ).

1, caudal liver primordium; 2, cranial liver primordium; 3, fore-gut; 4, sinus venosus; 5, right omphalomesenteric vein; 6, anterior intestinal portal; 7, notochord; 8, dorsal wall of fore-gut; 9, yolk sac.

instead of the primary anlage and is present in the 16- to 24-somite chick (*Keibel and Abraham, 1900; Kingsbury, Alexanderson, and Kornstein, 1956*) and the 25-somite lapwing (*Vanellus vanellus*) embryo (*Grosser and Tandler, 1909*). The ventral or caudal primordium usually becomes recognizable somewhat later (cf. Fig. 202); thus it has been observed in the chick of 19 to 25 somites (*Keibel and Abraham, 1900; Kingsbury, Alexanderson, and Kornstein, 1956*), the lapwing (*Vanellus vanellus*) of 27 somites (*Abraham, 1901*), and the duck (*Anas platyrhynchos*) of 29 to 30 somites (*Hildebrandt, 1902*). According to the observations of Weber



(1902b), the caudal diverticulum may appear in the duck at the 23-somite stage and the cranial diverticulum not until the 28-somite stage.

In the chick, the cranial liver diverticulum is usually narrow and cylindrical in the beginning (Goette, 1867; Duval, 1889; Felix, 1892; Hammar, 1893; Brouha, 1898a, 1898b), although it may be flat (Shore, 1891; Choronshtitzky, 1900). In the red-winged blackbird (*Agelaius phoeniceus*), and probably in the sparrow (*Passer domesticus*) as well, it is an insignificant outgrowth (Elias, 1955). The caudal liver primordium is wider than the

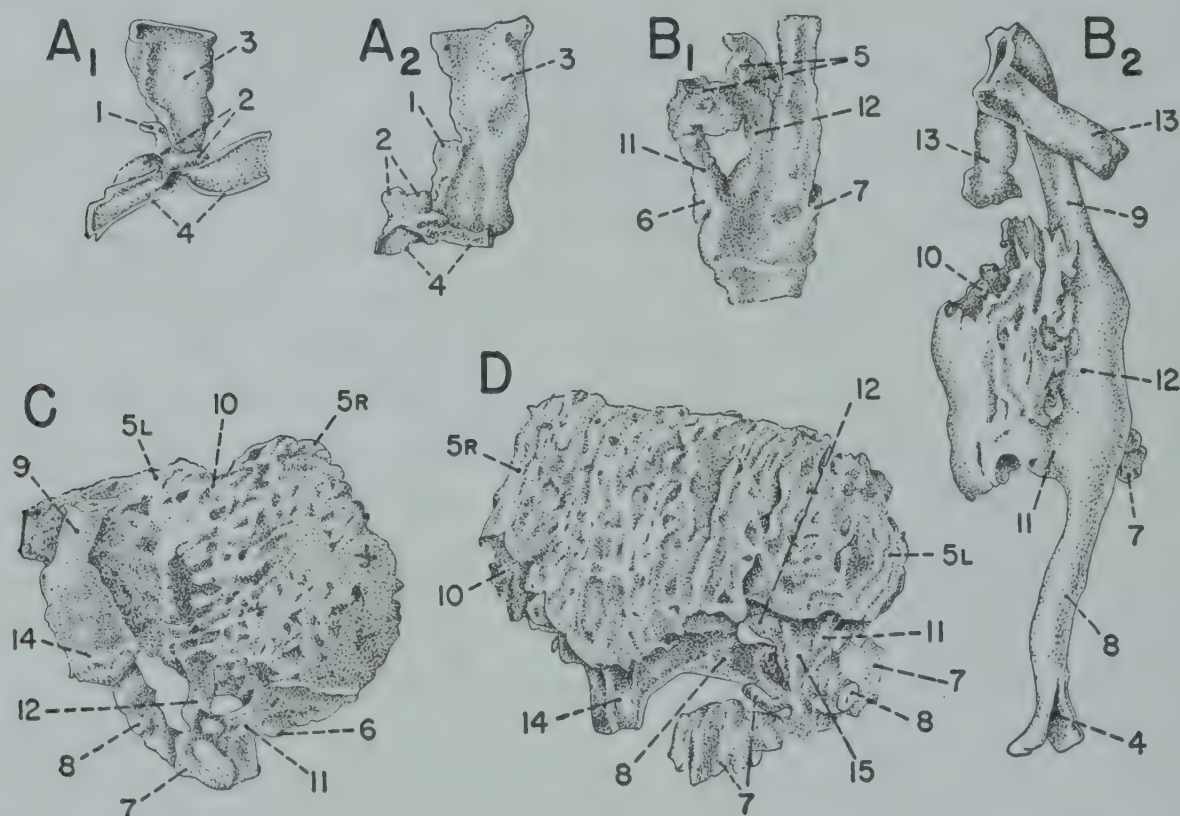


Fig. 203. Reconstructions of the chick embryo's liver at various stages of development. (Redrawn with modifications after Hammar, 1893.)

A<sub>1</sub>, ventral, and A<sub>2</sub>, left ventral view, during the third day of incubation; B<sub>1</sub>, view from left side at the beginning of the fourth day; B<sub>2</sub>, left ventral view late in the fourth day; C, right dorsal view during the fifth day; D, left view during the sixth day. All  $\times 30$ .

1, cephalic liver diverticulum; 2, caudal liver diverticulum; 3, fore-gut; 4, anterior intestinal portal; 5, hepatic tissue; 6, gall bladder; 7, pancreas; 8, duodenum; 9, esophagus; 10, location of ductus venosus; 11, caudal liver duct; 12, cranial liver duct; 13, lung primordium; 14, gizzard; 15, common bile duct.

cranial primordium (Fig. 203-A<sub>1</sub> and A<sub>2</sub>) from the beginning (Goette, 1867). It may be incompletely divided in two by a median furrow (Hammar, 1893).

### The Gross Development of the Liver

The secondary liver primordia rapidly extend themselves forward in the ventral mesentery during the first part of the chick's third day of incubation (Fig. 204-B). The caudal diverticulum becomes wider and flatter as it grows forward (Felix, 1892; Hammar, 1893; Brouha, 1898a, 1898b). On the right side (Fig. 202-F<sub>3</sub>), it also grows caudad ventral to the right vitel-



line vein (Goette, 1867; Felix, 1892; Brouha, 1898b). The gastroduodenal part of the gut is now curving to the left, so that the liver diverticula and the ductus venosus are beginning to be displaced to the right of the gut.

By the middle of the chick's third day of incubation, tissue has been proliferated laterally from the distal part of the cranial diverticulum, and both diverticula are now flat plates, luminate proximally (Goette, 1867; Felix, 1892; Hammar, 1893; Choronshtzky, 1900; Elias, 1955). Small evaginations from the wall of the ductus venosus can be seen projecting

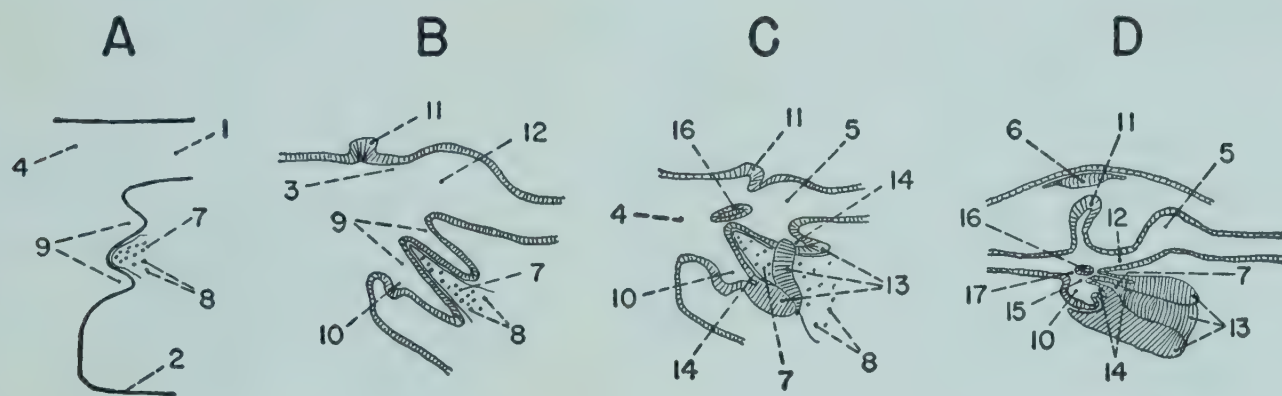


Fig. 204. The development of the liver of the chick (*Gallus gallus*) embryo between the third and the fifth day of incubation, as shown by schematic longitudinal sections. (Redrawn with modifications after Choronshtzky, 1900.)

A, early in the third day; B, during the latter half of the third day; C, at the end of the third day or early in the fourth day; D, early in the fifth day. In C and D, structures to the right of the midline have been projected upon the section.

1, fore-gut; 2, yolk stalk; 3, intestine; 4, mid-gut; 5, stomach; 6, spleen; 7, sinus venosus; 8, blood cells; 9, secondary liver diverticulum; 10, gall bladder; 11, dorsal pancreas; 12, duodenum; 13, hepatic tissue; 14, biliary duct; 15, cystic duct; 16, right ventral pancreas rudiment; 17, common bile duct.

between irregularities on the medial surfaces of the plates (Choronshtzky, 1900); these evaginations are early capillary sprouts.

During the second half of the third day (Fig. 205-A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, and A<sub>4</sub>), endodermal outgrowths are proliferated from the liver plates on all sides of the ductus venosus, as well as dorsal and ventral to the caudal part of the sinus venosus, ventral to the cranial end of the right vitelline vein, and radially into the surrounding connective tissue (Brouha, 1898b). The proliferation of liver trabeculae is accompanied by the sprouting of capillaries from the ductus venosus (Goette, 1867; Shore, 1891; Frobeen, 1892; Hammar, 1893; Minot, 1900b). The capillaries interdigitate (Elias, 1955) with the endodermal proliferations in a continually more complex fashion, as new trabeculae and new capillaries sprout from existing ones, especially at the periphery of the liver (Shore, 1891).

Soon trabeculae of tissue derived from both liver plates fuse with one another lateral to the ductus venosus (Fig. 203-B<sub>1</sub>) and thus form anastomoses between the cranial and caudal plates (Goette, 1867; Shore, 1891; Felix, 1892; Hammar, 1893; Elias, 1955). In the chick, the first anastomoses



may occur before the end of the third day (*Shore, 1891; Elias, 1955*) or early in the fourth day (*Hammar, 1893*); in the duck (*Anas platyrhynchos*), they are seen after 86 to 90 hours' incubation (*Weber, 1902b*). By this time, the progressive caudad closure of the gut has moved the liver rudiment from the region of the anterior intestinal portal into the gut proper.

During the course of the chick's fourth day of development, the lateral anastomoses become so numerous that a network of liver tissue forms an open-meshed cylinder around the ductus venosus (Fig. 203-B<sub>2</sub>). The

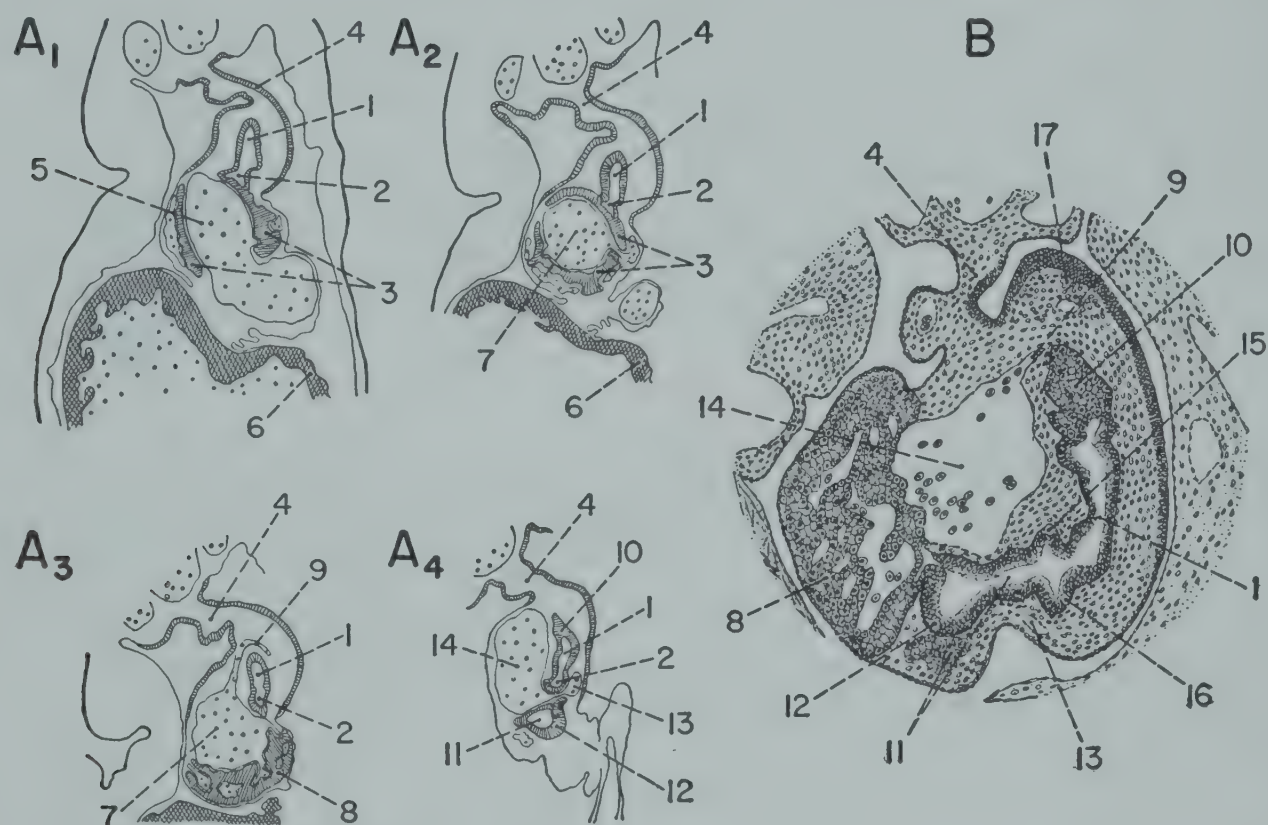


Fig. 205. Stages in the development of the liver in the chick embryo, between the middle of the third and the middle of the fourth days of incubation. (Redrawn with modifications after Choronshtitzky, 1900.)

A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, and A<sub>4</sub>, cross sections taken at successively more posterior levels of an embryo incubated 3.5 to 4 days; B, section through the caudal portion of the liver, the gall bladder, and the pancreatic primordia of an embryo incubated 4 to 4.5 days.

1, gut; 2, cranial hepatic diverticulum or duct; 3, tissue proliferated from cranial hepatic diverticulum; 4, mesentery; 5, sinus venosus; 6, heart; 7, ductus venosus; 8, tissue proliferated from caudal hepatic diverticulum; 9, splenic vein; 10, dorsal pancreas; 11, caudal hepatic diverticulum or duct; 12, gall bladder; 13, left vitelline vein; 14, right vitelline vein; 15, right ventral pancreas; 16, left ventral pancreas; 17, anlage of spleen.

dorsal part of this cylinder extends farther craniad than the ventral part; the latter, however, extends farther caudad than the dorsal part (*Hammar, 1893; Choronshtitzky, 1900*). On the next day (Fig. 205-A<sub>4</sub>), the liver is smooth externally and has the appearance of a compact organ, although its interior is still trabecular (*Froben, 1892*).

According to *Hammar (1893)*, the left lobe of the liver develops from the left side of the cylinder of liver tissue, and the right lobe arises from the remainder of the cylinder (Fig. 203-C and D). As *Brouha (1898b)* noted, however, the division of the liver into a right and a left lobe is



fortuitous and is due to the fact that the growing liver merely occupies the space available to it. Actually, the liver consists of anterior, posterior, and intermediate portions.

The anterior part of the liver develops around the most caudal part of the sinus venosus and immediately behind it and spreads transversely to fill the body cavity from side to side. Early in the chick's fourth day of incubation, the liver grows forward on the left side of the ductus venosus until it surrounds the mouth of a medial branch of the anterior part of the left umbilical vein, recently given off to the ductus venosus (see Chapter 8). This branch now sends ramifications among the liver trabeculae. On the right side, the liver has not yet reached the corresponding branch of the right umbilical vein (*Brouha, 1898b*). A comparable stage is seen in the red-winged blackbird (*Agelaius phoeniceus*) during the latter half of the third day of incubation, when a solid mass of cells, derived entirely from the caudal liver bud, grows forward between and beneath the vitelline veins and surrounds the mouth of the left duct of Cuvier, which empties directly into the sinus venosus at this time (*Elias, 1955*).

By the middle of the chick's fourth incubation day, the terminal (anterior) segments of both umbilical veins have atrophied, and the veins empty into the ductus venosus through their former medial branches. Blood passing through the left vein must therefore traverse the vascular network of the left anterior part of the liver, which now begins to grow at an accelerated rate as a result of its increased blood supply. By the middle of the fifth day, it has reached the left body wall. Through the great increase in the thickness of this region of the liver, a deep indentation has been passively created on the dorsal surface of the liver, slightly to the left of the midline, at the point where the enterohepatic mesentery (ventral mesogastrium) is attached. The presence of this indentation gives the anterior part of the liver the appearance of being divided into two halves, or lobes. In passing caudad, the left lobe gradually becomes smaller as the mesentery becomes broader, and it finally gives way to the mesentery about at the midlevel of the liver. The right lobe of the liver, through which the ductus venosus passes, extends to the right body wall; like the left lobe, it is inserted between the body wall and the digestive tube and is suspended by a lateral (accessory) mesentery. Liver tissue has now advanced cranial to the new mouth of the right umbilical vein, which has decreased in diameter. Part of the hepatic vascular network has condensed into a channel forming a new terminal segment for the left umbilical vein. The mouths of both umbilical veins are now on the ventral side of the ductus venosus.

The right lobe of the liver continues posteriorly as the intermediate portion of the liver, which develops around the ductus venosus and lies in the right side of the body cavity after the middle of the third day. Before the end of the fourth day, this part of the liver undergoes a rotation which



turns its originally dorsal surface to the left, so that the attachment of the enterohepatic mesentery is shifted from the left end of the dorsal surface to the ventral end of the left lateral surface. Early in the fifth day, the intermediate portion of the liver, at the level where it merges with the posterior portion, invades the left side of the abdominal cavity.

The posterior part of the liver develops around the right vitelline vein. Between the middle and end of the third incubation day, liver tissue grows caudad beneath this vein and spreads dorsad over its entire right surface. The atrophy of the left vitelline vein (Fig. 205-A<sub>4</sub>) and consequent obliteration of the first peri-intestinal venous ring (see Chapter 8) now incorporates the right vein into the ductus venosus. From the fourth to the seventh day, inclusive, the liver, bulging into the right half of the body cavity, extends itself progressively farther caudad along the right side of the ductus venosus to the point where the latter turns dorsad to the site of the former dorsal anastomosis between the vitelline veins. From the right lateral part of the liver, a free lobe then grows posteriorly in the body cavity.

The liver's growth rate is highest, of course, during the early stages of its development, as is indicated by relative weight increments. The tabulation, compiled from the data of Schmalhausen (1926), Tamiya (1927b),

Age of Embryo (days)	Wet Weight of Liver (mg.)	Dry Weight of Liver (mg.)
4	0.3	
5	1.8	0.121
6	5.1	0.541
7	11.0	0.807
8	17.4	1.175

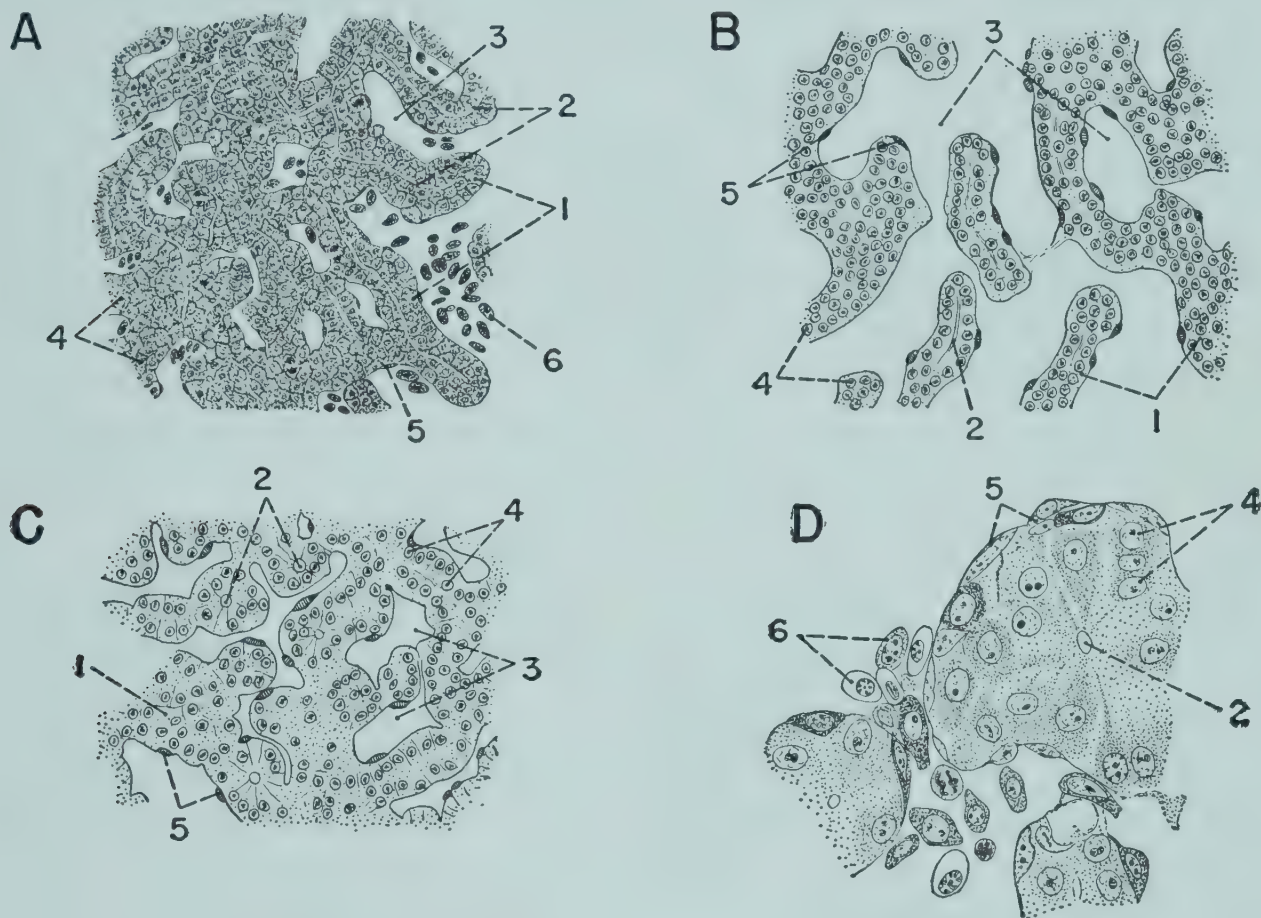
and Dumm and Levy (1949), shows the increase in the wet and dry weight of the chick embryo's liver from the fourth to the eighth day of incubation, inclusive. After the eleventh or twelfth day, when the liver weighs from 75 to 100 mg. (Schmalhausen, 1926; Duyff, 1939; Dumm and Levy, 1949; Herrmann and Barry, 1955), the growth rate slows rather abruptly (Duyff, 1939), and the ratio of liver weight to body weight also decreases (Dumm and Levy, 1949). The increments in absolute weight are so large, however, that the liver weighs 0.58 to 0.65 gm. by the nineteenth or twentieth day (Schmalhausen, 1926; Dumm and Levy, 1949; Herrmann and Barry, 1955) and 0.82 to 1.5 gm. by the hatching date (Latimer, 1925; Romanoff, Smith and Sullivan, 1938; Fronda and Marcelo, 1938; Herrmann and Barry, 1955). Incubation at reduced or elevated temperatures (such as 36.5° C. and 38.5° C.) retards the growth of the liver, especially during the last days of development; at hatching, the ratio of liver weight to body weight is lower than normal (Romanoff, Smith, and Sullivan, 1938). If liver fragments from the 6-day embryo liver are grafted on to the area vasculosa



on the fourth day, the weight of the host's liver may increase to two or three times normal within a few days after the graft is implanted (Weiss and Wang, 1941; Weiss, 1947). The injection of guinea pig antisera against adult chicken liver has a similar though less pronounced effect (Weiss, 1939, 1947).

### Histological and Functional Development of the Liver

The majority of observers have indicated that the first endodermal outgrowths arising from the secondary liver diverticula are solid cords or



**Fig. 206.** Histological structure of the liver of the chick embryo at various stages of development. (Redrawn with modifications A, after Frobeen, 1892; B and C, after Minot, 1900b; D, after Haff, 1914.)

A, at 96 hours ( $\times 125$ ); B, at 6 days ( $\times 150$ ); C, at 11 days ( $\times 150$ ); D, at 7.5 days ( $\times 300$ ).

1, liver trabeculae; 2, bile duct; 3, liver sinus; 4, nuclei of liver parenchymal cells; 5, endothelial cells of sinuses; 6, blood cells.

trabeculae in the chick (Shore, 1891; Felix, 1892; Frobeen, 1892; Choronshtzky, 1900; Minot, 1900b) as in passerine birds (Elias, 1955). There is also agreement that the liver parenchyma of the 4-day chick consists of tubules, each with a small lumen, or bile capillary (Fig. 206-A). According to Elias (1955), the chick's hollow secondary liver diverticula extend themselves from the beginning as tubules, which can be identified as such in the 72-hour chick (whereas the solid proliferations seen in passerine birds do not become luminate until some time after the third day of incubation). It has also been said that some of the buds proliferated by the chick's



secondary diverticula are solid and some luminate (*Kingsbury, Alexander, and Kornstein, 1956*). Haff (1914) stated that the trabeculae contain lumina by the middle of the chick's fourth day, at a time when Froben (1892) found them to be solid. Shore (1891) noted the presence of lumina in all the hepatic cylinders of the 90-hour chick except those newly formed at the periphery of the liver, as if the formation of lumina were beginning centrally and progressing outward.

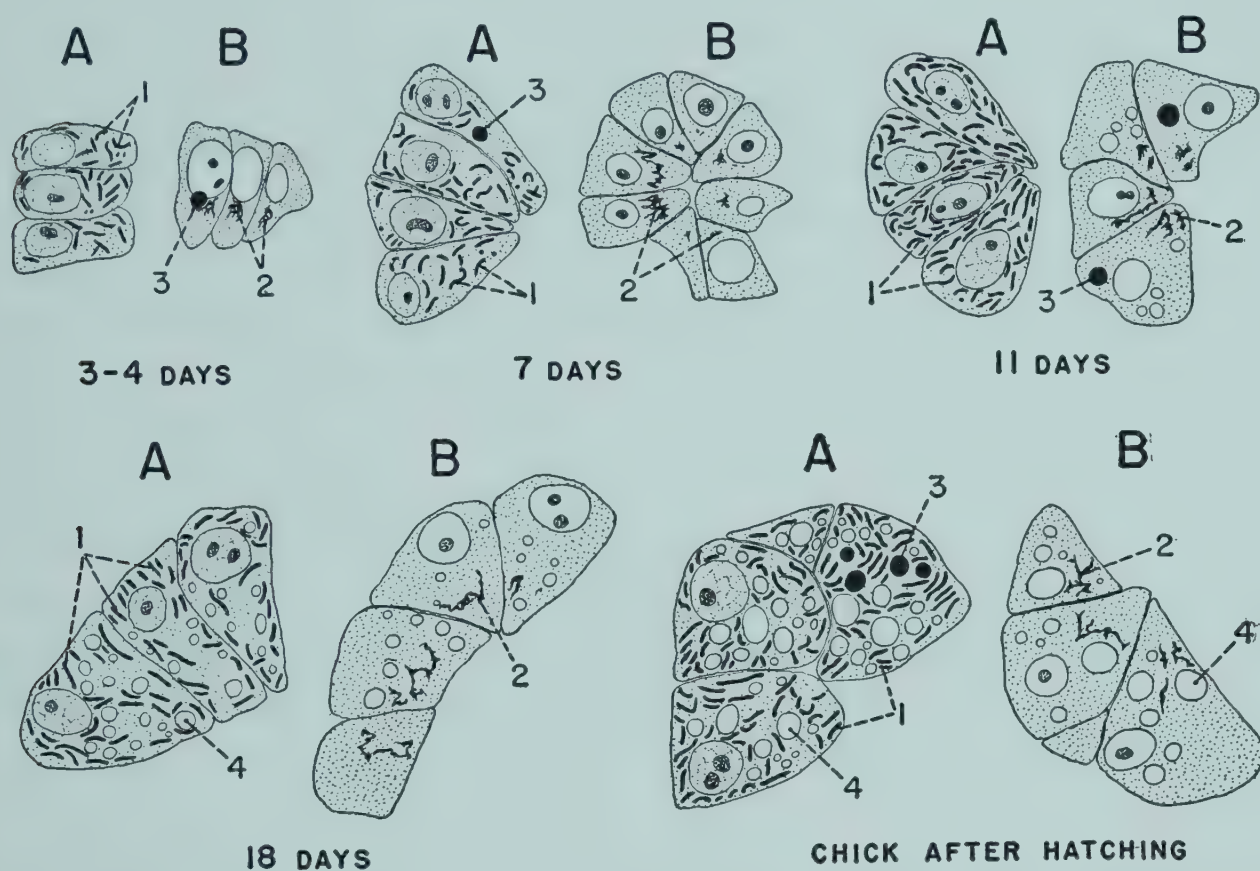
In cross section, each tubule is seen to consist of four to eight cells arranged radially around a bile capillary (Fig. 206-D); the nuclei are in a peripheral position. The tubules or plates are two cells thick in the chick; in passerine birds, they thin out to one layer of cells (*Elias, 1955*).

The endothelium of the blood capillaries, or sinusoids (*Minot, 1900b*), of the liver is closely applied to the surface of the trabeculae during most of the embryonic period. At the periphery of the 4- to 5-day chick embryo's liver, some mesenchymal tissue intervenes between the trabeculae and the capillary endothelium, which loosens in this region and merges imperceptibly with the mesenchyme (*Haff, 1914*). The sinusoids are narrow in the beginning, but they gradually become wider (Fig. 206-B) until the fifth or sixth day (*Remak, 1855, pp. 51-53; Froben, 1892; Minot, 1900b*). During the sixth day, the extravascular mesenchymal tissue disappears, and the loose mesenchyme peripheral to the liver (which is rapidly increasing in size) thins to a single layer of flat peritoneal cells with basophilic cytoplasm and large, chromatin-poor nuclei, each containing one large nucleolus. On the seventh and eighth days, the endothelial walls of the sinusoids are replaced by a loose-meshed connective tissue reticulum proliferated by the endothelial and peritoneal cells, first in the peripheral portions of the liver and then in the more central portions. The cells of this reticulum differentiate to form blood cells (cf. Fig. 206-D), predominantly erythrocytes (see Chapter 8). On the ninth day, the reticulum disappears and the endothelium is again closely applied to the trabeculae (*Haff, 1914*). At this time, Kupffer cells (stellate phagocytic cells derived from the endothelium) are unmistakably demonstrable (*Okkels, 1930*). The sinusoids now begin to decrease in diameter (Fig. 206-C) as the liver parenchyma increases in density; eventually, they become as narrow as capillaries. On the eleventh day, the perivascular reticulum reappears (*Haff, 1914*) and participates in granulopoiesis, the intensity of which is greatest on the fourteenth or fifteenth day (see Chapter 8). Collagen, the specific cell protein of connective tissue, does not undergo a significant increase until the eighteenth day (*Herrmann and Barry, 1955*).

As Fig. 207 shows, the cells of the liver parenchyma undergo a marked increase in size between the chick's fourth and sixth days; a second increase after the twelfth day is probably correlated with the accumulation of lipoid inclusions in the cytoplasm (*Dalton, 1934; Trotti, 1934*). The mitochondria



of the parenchymal cells are few and filamentous on the third day, numerous and short by the sixth day. From this time until the eighteenth day, they increase in length and number. They align themselves parallel to the long axis of the cell on the eighth day (*Dalton, 1934*), although they have been found arranged radially on the twelfth day (*Waugh, 1942*). After the eighteenth day, they decrease somewhat in length. Sulfonamides injected into the 12-day incubated egg cause the mitochondria to be transformed into globules in 8 to 12 hours (*Waugh, 1942*). The Golgi apparatus of the hepatic cell is situated between the nucleus and the bile capillary. On the fourth day, it is a tightly woven network lying near the nucleus;



**Fig. 207.** Hepatic cells from the liver of the chick embryo at successive stages of development. (Redrawn with modifications after *Dalton, 1934*.)

Special staining methods reveal in A, mitochondria, and in B, the Golgi network. All  $\times 600$ .

1, mitochondria; 2, Golgi network; 3, fat globule; 4, cholesterol droplet.

from this time until the sixteenth or eighteenth day, it undergoes an enormous expansion and moves away from the nucleus and closer to the bile capillary (*Dalton, 1934*). The Golgi apparatus becomes severely fractured and disorganized 24 hours after the injection of sulfonamides into the allantoic sac (*Waugh, 1942*).

Lipoid globules appear in the parenchymal cells after 6 days (*Hanes, 1912; Kingsbury, Alexanderson, and Kornstein, 1956*) to 8 days (*Kirkman, 1931*) of incubation and increase in size and number until the end of development (Fig. 207). They are especially numerous in regions close to blood vessels (*Trotti, 1934*). In each cell, the fat droplets accumulate between the nucleus and the bile capillary (*Kirkman, 1931*) and eventually



occupy so much space that the cytoplasm appears as trabeculae between the fat spheres (*Trotti, 1934*). Various chemical and physical properties indicate that the fat droplets consist at first of a mixture of lipoids, with phosphatides predominating (*Hanes, 1912*). Between the eleventh and fourteenth days, some of the globules become birefringent, or anisotropic (*Hanes, 1912; Dalton, 1937a*). By the seventeenth day, all the fat spheres are anisotropic (*Hanes, 1912*) and are probably formed of free cholesterol, cholesterol esters, and mixtures of cholesterol and fatty acids (*Hanes, 1912; Dalton, 1934, 1937a*). It may be noted that anisotropic globules appear in grafts of the 3-day chick embryo's liver 4 days after explantation to the chorioallantois of 16-day-old embryos; it therefore appears that the parenchymal cells are capable of cholesterol esterification and storage when their total age is only 7 days (*Dalton, 1937a*). The observed degeneration of grafted liver cells upon reaching a total age of 12 days (*Sandstrom, 1934*) is possibly due to the incompatibility of conditions in a graft with the functional state of the cells (*Dalton, 1937a*). The introduction of sulfonamides into the egg on the twelfth incubation day greatly accelerates the appearance of fat in the hepatic cells, apparently by unmasking latent fat, since the total amount of fat in the liver does not increase (*Waugh, 1942*).

Simultaneously with the onset of cholesterol storage, the fat content of the liver, as percentage of wet weight, begins to rise at an accelerated rate (*Dumm and Levy, 1949*). At the same time, the red or reddish-brown color of the liver starts to acquire a yellowish tint (*Weber, 1850; Romanoff, 1951b*), which increases until the end of incubation, when the liver is a bright orange-yellow (*Weber, 1850; Shore, 1891; Hanes, 1912; Romanoff, 1951b*). This color change is probably due to the transport of yellow pigments from the yolk, along with lipids (*Kingsbury, Alexanderson, and Kornstein, 1956*).

Bile secretion has been observed in chick embryos incubated 7 to 9 days (*Kuo, 1932a*). The injection of a bile salt (sodium dehydrocholate) into the chicken egg on the twelfth day of incubation stimulates bile secretion. So also does the injection of cortisone, but it is not known whether the effect of cortisone is direct or indirect (*Mosbaugh and Ham, 1951*).

It is probable that the liver is capable of storing glycogen on the chick's seventh incubation day, for appropriate stains may reveal the presence of glycogen in a few parenchymal cells at this time, or even on the sixth day. The amount of glycogen gradually increases until the ninth day and then decreases until the twelfth day. During this entire period, glycogen occurs principally in cells near large veins, but it becomes more evenly distributed throughout the liver after the fourteenth day (*Dalton, 1937b*) as its concentration rapidly rises (*Potvin and Aron, 1927*), the total glycogen content of the liver being 0.66 per cent on the fourteenth day and 2.34 per cent on the nineteenth day (*Wladimiroff, 1930*). Sulfonamides injected into



12-day embryos stimulate glycogenesis for 6 or 8 hours, but glycogen then disappears entirely from the cells (*Waugh, 1942*).

Peptidase activity in the liver of the chick embryo is sixteen times as great on the eighth day of incubation as it is on the fifth day. By the fourteenth day, it has increased to twenty times its 5-day value (*Dumm and Levy, 1949*). Quinine oxidase activity, on the other hand, diminishes during incubation; on the twentieth day, it is only about 70 per cent as high as on the tenth day (*Marshall, 1945*).

During the period of the liver's most rapid growth, that is, from the chick's fifth to eighth incubation days, inclusive, the respiratory rate (oxygen quotient) of the organ changes but little and is not greatly different from that of the adult liver; the rate of anaerobic glycolysis (in chicken serum), however, decreases from +16.5 to +7.2, the latter figure being at least three times as high as the rate in the adult liver (*Tamiya, 1927b*).

#### *The Development of Liver Tissue in Vitro*

Like other tissues, the liver of the chick embryo can be cultivated *in vitro* in artificial media. The cultural characteristics vary somewhat with embryonic age, the age of the culture, and the nature of the medium.

If derived from an embryo not more than 11 days old, liver fragments can grow in a nonnutritive salt solution (*Heaton, 1926*); the addition of dextrose and protein (bouillon) to the medium increases the age limit to 15 or 16 days (*Lynch, 1921*), and the further addition of plasma extends it beyond the embryonic period (*Nordmann, 1929*). Embryonic extract in the medium exerts its favorable effect on the liver as on other cultured tissues.

In general, three types of cells appear in cultures of the embryonic chick liver (*Lynch, 1921; Nordmann, 1929; Okkels, 1929; Bisceglie, 1931*). These are parenchymal cells; mesenchymal cells, including the endothelial cells of the liver sinusoids and cells derived from the mesothelial capsule; and macrophages or wandering cells, most of which are doubtless also of mesenchymal derivation, although some may not be.

The mesenchymal elements are the first to appear (*Bisceglie, 1931; Frederic, 1950a, 1952*). In cultures from the 8-day chick's liver, they grow out within the first 24 hours after explantation, migrating radially from the explant in the form of a loose-meshed reticulum. This reticulum is composed largely of endothelial cells but may contain some mesothelial elements resembling fibroblasts (*Kapel, 1926, 1929; Ephrussi, 1930*); the latter are much more numerous, however, in cultures explanted after the twelfth day of incubation (*Bisceglie, 1931*). The parenchymal cells migrate from the explant after 40 or 48 hours of incubation (*Nordmann, 1929; Bisceglie, 1931*) in the form of a continuous epithelial membrane. This membrane may be at a different level from the mesenchymal reticulum



(Nordmann, 1929; Bisceglie, 1931), which, of course, extends peripheral to it. In media containing embryonic extract, the endothelial cells may proliferate so intensely that they soon overgrow the entire culture (*Doljanski, 1929a, 1931; Bisceglie, 1931*). The superior vitality of the endothelial cells is also shown by their ability to grow in culture when explanted as long as 6 hours after the experimental death (by exsanguination) of the embryo, whereas the parenchymal cells survive for only 4 hours (*Wilburg, 1937*).

Both epithelial and endothelial cells exhibit a progressively lower growth potential with advancing embryonic age. In cultures of 19- or 20-day livers (in media lacking embryonic extract), initial cellular migration may be delayed until 4 or 5 days after explantation (*Nordmann, 1929*). Mitotic activity also declines. The tabulation reveals how the mitotic indexes (determined during the first days of culture in media containing embryonic extract) decrease as the age of the donor embryo increases (*Bisceglie, 1931*).

Embryonic Age (days)	Mitotic Index of Epithelial Cells (per cent)	Mitotic Index of Endothelial Cells (per cent)
5	11.30	18.43
9	10.87	17.27
14	9.36	16.92
18	8.93	15.81

Pure cultures of hepatic epithelial cells may be obtained when the growth of the endothelial cells is suppressed by the use of diluted or heat-inactivated embryonic extract (*Doljanski, 1929a, 1931; Bisceglie, 1931*). Such pure cultures can be maintained for prolonged periods of time, perhaps indefinitely, without much change in cellular morphology. The growth rate, as shown by the mitotic index, decreases in the first four subcultures, then rises during the fifth or sixth passage to equal or surpass the original rate; thereafter, the mitotic index is stabilized at a high level which is entirely independent of embryonic age at the time of explantation (*Bisceglie, 1931*). The most favorable medium for pure cultures of hepatic epithelium is one that contains only 20 per cent embryonic extract diluted with Tyrode's solution (*Doljanski, 1931*).

The epithelial membrane of liver cultures is formed of large, coherent polygonal elements each with a round, intensely staining nucleus containing one or two nucleoli (*Doljanski, 1929a, 1929b, 1931; Nordmann, 1929; Okkels, 1929*). The mitochondria are very numerous and vary in size and shape (*Lynch, 1921; Bisceglie, 1931; Doljanski, 1931; Frederic, 1950a*); after a few days of culture, short rodlike elements tend to become filamentous (*Frederic, 1950a*). The mitochondria may be masked by cytoplasmic granules, identified by Lynch (1921) as bile granules which appear after 2 days of culture; Frederic (1950a, 1952) found similar granules



grouped around the nucleus and, from their appearance under the contrast phase microscope, concluded that they correspond to certain basophilic granules rich in ribonucleic acid. After 5 or 6 days of culture, sulfhydryl substances are no longer distributed uniformly in the cytoplasm but are concentrated in large granules near the nucleus (*Frederic, 1950a, 1952*). Glycogen appears in the cells either dissolved in the cytoplasm or in the form of granules, droplets, or masses which may be concentrated near the nucleus or near the cell membrane or may be distributed throughout the cytoplasm (*Doljanski, 1929b, 1930b; Nordmann, 1929*). In untransplanted cultures, the amount of glycogen decreases from day to day and finally disappears. The rapidity of its disappearance varies directly with embryonic age at the time of explantation; thus a reduction in glycogenic function appears to accompany the decline in growth potential (*Nordmann, 1929*). Glycogen also diminishes in amount with each successive transplant; it is never present in actively proliferating pure cultures (*Doljanski, 1930b, 1931*), a fact which may indicate an antagonism between functional activity and cellular proliferation (*Doljanski, 1930b*) or may signify the physiological dedifferentiation of the hepatic cells (*Bisceglie, 1931*). The quantity of fat in the cells is correlated positively with the age of the embryo from which the explant is taken but not with the age of the culture, if the latter is untransplanted (*Lynch, 1921*). When subcultures are made, however, the accumulation of fat increases progressively as the glycogen disappears (*Doljanski, 1929b, 1931*).

The endothelial cells are slender, fairly transparent elements each containing an elongated, pale-staining nucleus with two nucleoli. The mitochondria have been described as few in number and situated near the nucleus (*Nordmann, 1929*), and also as rather long undulating filaments disposed parallel with the longitudinal axis of the cell (*Bisceglie, 1931*). The cytoplasm, otherwise rather structureless (*Doljanski, 1930a*), is drawn out into processes which anastomose with the processes of neighboring cells. The meshes of the reticulum formed in this manner may be round or oval, or may consist merely of narrow fissures (*Bisceglie, 1931*).

The endothelial cells are easily isolated in pure culture because of their prolific growth and peripheral position. If incubated in liquid media, they retain their structural characteristics, but if cultivated in media containing plasma, they resemble fibroblasts after a few passages (*Doljanski, 1930a, 1931; Ephrussi, 1930; Bisceglie, 1931*). The formation of collagen fibers, which is not a constant phenomenon (*Bisceglie, 1931*), may indicate an actual transformation of endothelial elements into fibroblasts (*Ephrussi, 1930*), or merely the admixture of fibroblasts (mesothelial cells) with the endothelial cells (*Bisceglie, 1931*). Upon continued culture, many of the peripheral cells detach themselves, become amoeboid, and assume the typical characteristics of macrophages or clasmatoocytes (*Ephrussi, 1930*;



*Bisceglie*, 1931); simultaneously, the culture begins to exhibit fibrinolytic properties (*Kapel*, 1926; *Doljanski*, 1931). The amoeboid elements rapidly increase in number. After a few transplantations, the entire culture may consist of macrophages (*Ephrussi*, 1930). The origin of these cells is uncertain; they may be derived from Kupffer cells included in the original endothelial culture (*Bisceglie*, 1931), or from the endothelial cells, either directly (*Bisceglie*, 1931), or indirectly—that is, after their transformation into fibroblasts (*Ephrussi*, 1930), assuming that such a transformation occurs.

Free macrophages appear in mixed cultures of the embryonic chick liver in very variable numbers (*Okkels*, 1929; *Frederic*, 1950*b*). A cell of this type is generally round, except when sending out amoeboid prolongations; its nucleus is small, eccentric, and rich in chromatin, and its cytoplasm contains many fatty inclusions and neutral red granules (*Okkels*, 1929; *Doljanski*, 1930*a*; *Bisceglie*, 1931; *Frederic*, 1951). The Kupffer cells are probably one source of the macrophages in this type of culture (*Doljanski*, 1930*a*; *Bisceglie*, 1931). According to *Doljanski* (1930*a*), small, isolated groups of Kupffer cells may become incorporated into the epithelial membrane derived from the hepatic parenchyma; these cells, which resemble fixed wandering cells, detach themselves and become free macrophages when they find themselves at the periphery of the epithelial membrane. An epithelial origin of macrophages has also been suggested (*Kapel*, 1926, 1929), and, in fact, the transformation of peripheral epithelial cells into macrophages has been reported (*Frederic*, 1950*b*, 1951).

### The Development of the Gall Bladder and the Biliary Ducts

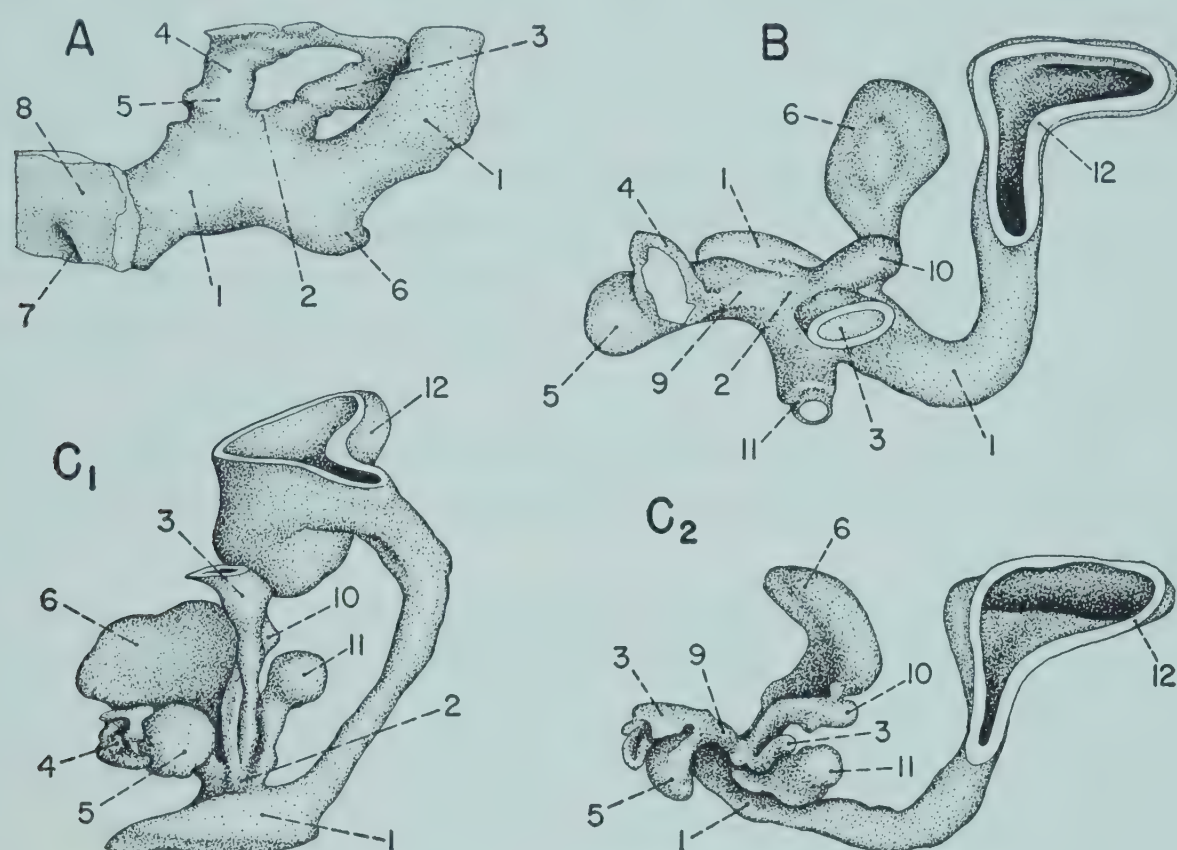
While the distal portions of the secondary hepatic diverticula are giving rise to the liver, the hollow proximal portions are undergoing changes in orientation and are differentiating into biliary ducts and the gall bladder. The ventral pancreatic ducts are in close proximity.

During the latter part of the chick's third incubation day, the elongating gut curves ventralward as it approaches the umbilicus, and its ventral wall, from which the hepatic diverticula take origin, is thus directed cephalad (*Brouha*, 1898*b*). About at this time, the gall bladder appears as an evagination of the ventral (originally caudal) wall of the caudal liver duct (*Goette*, 1867; *Brouha*, 1898*b*; *Choronshitzky*, 1900). The walls of the gall bladder are thick and consist of several layers of cylindrical cells (*Brouha*, 1898*b*). The primary liver diverticulum is now becoming constricted from the gut at either end (Fig. 208-A).

By the beginning of the fifth day, the primary hepatic diverticulum has decreased considerably in diameter and can be considered as equivalent to the ductus choledochus (common bile duct). The secondary diverticula are closer together and have become longer and narrower. The gall bladder



is beginning to be constricted from the caudal duct (Fig. 208-B; cf. Fig. 204-D). The duodenal loop is now beginning to form, and the portion representing the ascending limb (from which the liver ducts depend) has been carried ventralward and is directed from left to right and slightly craniocaudad (cf. Fig. 205-B). The primitive liver ducts therefore take origin from the wall of the gut that faces obliquely cranial and to the right (Hammar, 1893; Brouha, 1898b; Choronshtzky, 1900).



**Fig. 208.** Stages in the development of the biliary and pancreatic ducts, and of the gall bladder in the chick embryo; the liver trabeculae have been removed in all figures. (Redrawn with modifications after Brouha, 1898b.)

A, left lateral view at 68 hours; B, cephalic view at 100 hours; C<sub>1</sub>, ventral view, and C<sub>2</sub>, cephalic view at 124 hours. All  $\times 50$ .

1, intestine; 2, primary liver diverticulum; 3, cranial hepatic diverticulum (entero-hepatic duct); 4, caudal liver diverticulum (hepatocystic duct); 5, gall bladder; 6, dorsal pancreas; 7, anterior intestinal portal; 8, yolk sac; 9, cystic duct; 10, right ventral pancreas; 11, left ventral pancreas; 12, gizzard.

Early in the sixth day (Fig. 208-C<sub>1</sub> and C<sub>2</sub>), the orientation of the liver ducts is again altered, for the apex of the duodenal loop has been carried close to the ventral body wall, and the ascending limb of the loop, in passing from left to right, courses dorsad as well as slightly caudad. The caudal liver duct is thus brought to a position dorsal to and slightly to the right of the cranial duct. The gall bladder is becoming vesicular; its mouth has narrowed and has moved distally along the caudal duct, which has thereby been divided into a distal portion, or hepatocystic duct, and a proximal portion, or cystic duct. The cranial duct may now be termed the hepatic duct. By this time, the constriction of the common bile duct from the gut wall has reached the level of the ducts of the two ventral pan-



creatic primordia (which evaginated from the gut wall at the end of the fourth day), and the mouths of the ventral pancreatic ducts have therefore been shifted from the gut to the common bile duct.

By the middle of the seventh day, the division of the common bile duct into four parts has been continued to the gut wall; that is, the common bile duct no longer exists, and the biliary and pancreatic ducts enter the duodenum directly. The ascending limb of the duodenal loop now runs almost exactly ventrodorsad. The ducts, with the cystic duct dorsal to the hepatic duct, originate from the side of the duodenum that faces cephalad; later changes in the orientation of the duodenal loop bring the mouths of the ducts to the left ventral side of the gut (Goette, 1867; Felix, 1892). Between the sixth and the ninth days, in the chick (Saint-Remy, 1893) and the duck, *Anas platyrhynchos* (Weber, 1902c), the mouths of the biliary and ventral pancreatic ducts come to empty into the duodenum through a round papilla.

In the 7-day duck embryo, the gall bladder is an elongated structure. Its development takes place at the expense of its originally thick ventral wall (Weber, 1902b).

The differentiation of the mesenchymal portion of the biliary ducts is in its initial stages in chick embryos incubated 12 to 15 days. Within the condensed mesenchyme surrounding the endodermal epithelium, some of the cells are recognizable as myoblasts disposed in a thin circular layer. The nuclei of the myoblasts are oval and contain two nucleoli; the cytoplasm exhibits fine longitudinal striations and contains a chondriome consisting of uniformly distributed granules. In 17- to 18-day embryos, the myoblastic nuclei are elongated. A longitudinal muscle layer is beginning to appear. A muscular and a submucosal nerve plexus are demonstrable, as well as small ganglia situated near the intestine and the gall bladder (Staudacher-Dalle Aste, 1941).

The biliary ducts of 12- to 15-day chick embryos exhibit contractile activity when explanted to culture media at a temperature of 39° C. Contractions are weak at first but gradually become stronger and rhythmic, until, shortly after explantation, there are about two contractions per minute, each lasting 3 seconds. The speed of the contractile wave is about 150 mm. per minute. Explants from embryos aged 17 to 18 days exhibit three or four typically peristaltic contractions per minute; the duration of contraction is from 4 to 5 seconds, and the contractile wave travels only 30 to 40 mm. per minute (Staudacher-Dalle Aste, 1941).

## THE PANCREAS

In birds, the pancreas is an elongated gland that lies between the two limbs of the long, narrow duodenal loop and consists of a dorsal and a ventral lobe, which may be partially or entirely fused together or entirely



separate. A small, narrow process, known as the tail of the pancreas, extends from either or both of the principal lobes toward the spleen. Usually there are three excretory ducts leading from the pancreas into the ascending limb of the duodenal loop; the mouths of these ducts may vary somewhat in position but are usually in close proximity to those of the biliary ducts.

The pancreas of birds, like that of mammals, is a mixed gland whose internal and external secretions are elaborated by two separate and distinct types of tissue. Its exocrine portion is made up of cells arranged in small acini. These cells secrete digestive enzymes (amylase, protease, and lipase) and contain zymogen granules in varying abundance. The endocrine portion produces insulin (which assists in carbohydrate metabolism) and is composed of isolated cell groups, the islands of Langerhans. The insular tissue is more abundant in the dorsal than in the ventral lobe and is the predominating component of the juxtasplenic or caudal segment. In birds, the islands of Langerhans are of two types, dark and light. The dark islands contain cells that correspond to the so-called A- and D-cells of the mammalian pancreas, and the light islands contain B-cells, perhaps also D-cells. No C-cells occur in birds.

### Gross Development of the Pancreas

The pancreas arises from three primordia that appear and develop in close association with the liver anlagen. One of the pancreatic rudiments is a dorsal evagination of the gut; the other two are lateral evaginations known as the ventral pancreatic anlagen because of their position relative to the dorsal pancreas. The distal portions of these three evaginations proliferate masses of glandular tissue which fuse with one another to form the definitive pancreas. The proximal portions remain tubular and become the pancreatic ducts.

In the chick embryo, the dorsal pancreas becomes recognizable at stages of 22 to 31 somites, reached at incubation ages of 56 to 67 hours (*Saint-Remy, 1893; Keibel and Abraham, 1900*). Initially, it is a simple evagination of the dorsal wall of the gut, opposite the hepatic anlagen. The ventral pancreatic primordia may be represented at the same time by thickenings of the lateral walls of either the primary hepatic diverticulum or of one of the secondary hepatic diverticula, more often the cranial one. In the duck (*Anas platyrhynchos*), according to Weber (1902d), the dorsal pancreatic primordium exists at the stage of 22 somites in the form of discontinuous thickenings of the median dorsal and right dorsolateral wall of the open intestinal trough, posterior to the anterior intestinal portal; it is not incorporated into the closed gut until some time after the 28-somite stage, when the ventral primordia appear.

At the end of the chick's third day of incubation, the dorsal pancreas is a prominent dorsal evagination of the gut wall (cf. Fig. 208-A) located



immediately cranial to the dorsal anastomosis of the lateral vitelline veins (*Brouha, 1898b*). Its walls are thick and composed of actively proliferating tissue (*Saint-Remy, 1893*). During the fourth day, it grows out on a stalk, the mouth of which gradually narrows (*Felix, 1892*), and the ventral pancreatic buds cease to be simple expansions of the gut wall and become true evaginations (*Saint-Remy, 1893*). In the duck (*Anas platyrhynchos*) embryo, these stages are attained only a few hours later than in the chick (*Weber, 1902a*). Figure 209 shows the early development of the pancreas in the gull (*Larus sp.*) and the roseate tern (*Sterna dougalli*).

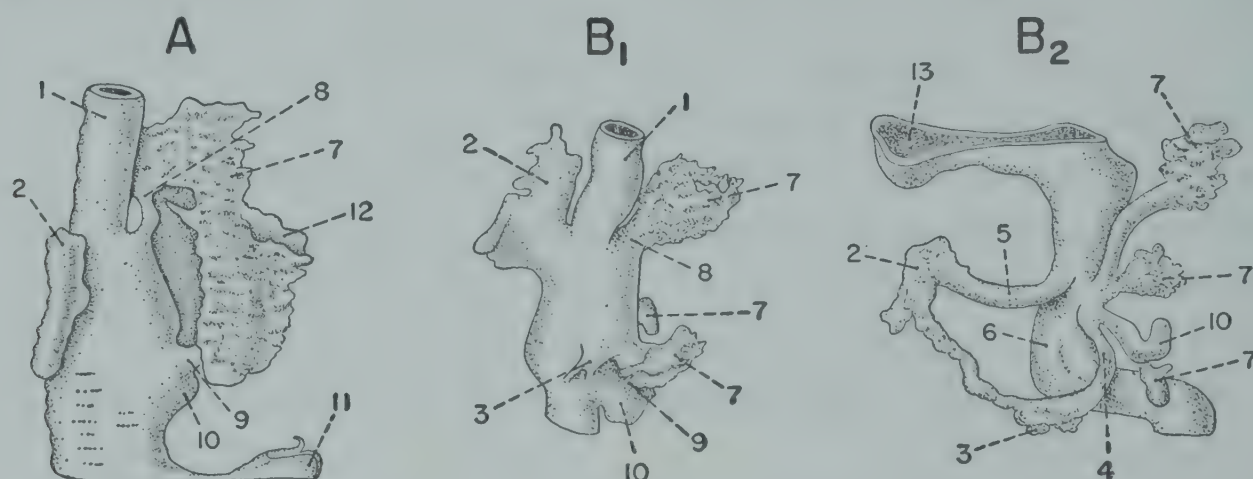


Fig. 209. Early development of the pancreas, shown by models from embryos. (Redrawn with modifications after Hammar, 1897a, 1897b.)

A, the roseate tern (*Sterna dougalli*) and B<sub>1</sub>, 1-mm. and B<sub>2</sub>, 10.5-mm. gulls (*Larus sp.*). Both  $\times 40$ .

1, alimentary canal; 2, dorsal pancreas; 3, ventral pancreas; 4, pancreatic duct (or duct of Wirsung); 5, Santorini's duct; 6, duodenum; 7, liver primordium; 8, cranial liver duct; 9, caudal liver duct; 10, gall bladder primordium; 11, yolk stalk; 12, sinus venosus; 13, ventricle.

In both the chick and the duck (*Anas platyrhynchos*), the dorsal pancreas begins to develop rapidly during the fifth day of incubation (cf. Fig. 208-B). It elongates in the dorsal mesentery along the left lateral surface of the ductus venosus (*Brouha, 1898b*), and its distal portion becomes a large mass of richly ramified glandular tubes (*Saint-Remy, 1893*; *Weber, 1902a*). The ventral primordia are less advanced; until the end of the fifth day, they are composed of short, nonramified epithelial tubules with a few small lateral buds (*Weber, 1902a*); then they begin to ramify (*Saint-Remy, 1893*). Meantime, with the incipient formation of the duodenal loop, the ventral pancreatic primordia are reoriented so that the right ventral pancreas is given off from the dorsal wall of the gut and the left ventral pancreas from the ventral wall (cf. Fig. 205-B; Fig. 208-B). The ventral pancreatic ducts empty into the duodenum close to the common bile duct, and the dorsal pancreatic duct runs directly ventralward to the left caudal wall of the duodenum (*Brouha, 1898b*; *Choronshitzky, 1900*).

Early in the chick's sixth day of incubation, at the time when the ducts temporarily empty into the common bile duct, the right and left ventral



pancreatic ducts originate, respectively, dorsal and ventral to the cranial hepatic duct; the right ventral duct lies obliquely to the left and the left ventral duct obliquely to the right (cf. Fig. 208-C<sub>2</sub>). They extend craniad and to the left and ramify distally into two glandular masses lying to the left of the cranial hepatic duct (cf. Fig. 208-C<sub>1</sub> and C<sub>2</sub>). The right ventral pancreatic mass is directly ventral to the dorsal pancreas and begins to fuse with it at this time, in both the chick (*Brouha, 1898b*) and the duck, *Anas platyrhynchos* (*Weber, 1902c*).

After the chick's sixth day, the dorsal pancreas enlarges much less rapidly than before (although it grows denser), and the ventral primordia begin to assume relatively greater importance (*Saint-Remy, 1893*). The dorsal pancreas develops between the two limbs of the duodenal loop, the right ventral pancreas grows along the descending (left ventral) limb of the loop, and the left ventral pancreas extends itself along the ascending (right dorsal) limb (*Giannelli, 1908*). The left ventral pancreas now begins to fuse with the right ventral pancreas (*Weber, 1902a; Giannelli, 1908*), which is already fusing with the dorsal pancreas, so that there is only one pancreatic gland by the middle of the seventh day (*Brouha, 1898b*); however, fusion of the ventral primordia is still not complete by the ninth day (*Saint-Remy, 1893*), when the pancreas extends to the apex of the duodenal loop (*Potvin and Aron, 1927*). Lastly, the left ventral pancreas fuses with the dorsal pancreas (*Weber, 1902a; Giannelli, 1908*). By the twelfth day, the pancreas has begun to acquire its definitive structure. At this time, tissue derived from the ventral primordia constitutes the larger part of the organ (*Saint-Remy, 1893*). The duct of the chick's dorsal pancreas is of smaller caliber than the ventral pancreatic ducts (*Giannelli, 1908*).

### Histological Development of the Pancreas

On the chick embryo's sixth day of incubation, the pancreas is composed of cellular cords, except in the caudal portion, where the gland consists of a compact mass of tissue with no trabeculation. The primitive pancreatic cells are small and polyhedral, with small nuclei, one or more nucleoli, and scanty cytoplasm. Beginning on the seventh day, the cellular cords gradually acquire lumina, which extend themselves from the center to the periphery of the glandular masses and are probably formed by the degeneration of the central cells of the trabeculae. During the second week of incubation, interstitial connective tissue and blood vessels penetrate among the trabeculae and tubules, but the growth of the parenchyma predominates during the last week of development (*Villamil, 1942*). The glandular tissue begins its differentiation, however, on the eighth day (*Potvin and Aron, 1927*), when it is possible to identify cells of the exocrine acini and of the dark and light endocrine islands (*Villamil, 1942*).



The acinic cells differentiate throughout the entire organ and are recognizable by certain nuclear changes (a reduction in the amount of chromatin and the appearance of one large, irregularly shaped nucleolus) and by their content of zymogen granules. These granules originate close to the nucleus but gradually shift to the apexes of the cells as lumina form in the solid trabeculae and the nuclei take up a peripheral position. The zymogen granules range in size from 0.724 microns to 1.448 microns on the chick's seventeenth day of incubation (Gage, 1945). They vary considerably in abundance during the last week of development and disappear if hydrochloric acid is introduced into the gizzard of the 18-day embryo (Flynn, 1942).

The first dark islands are seen in the "caudal" part of the gland (Gianelli, 1908; Villamil, 1942). Their differentiation is indicated by the appearance of A-cells. These cells have large oval nuclei with little chromatin and contain numerous small granules staining light red with azan. In the central cells of the dark islands, the granules are scattered through the cytoplasm; but in the peripheral cells, which border on capillaries, the granules are polarized toward the capillaries. As capillaries penetrate into the dark islands in increasing abundance, the cells become arranged in single rows around the capillaries, so that every cell is in intimate contact with a capillary and the granules of all the cells are polarized. A few primitive pancreatic cells that persist between the A-cells differentiate as D-cells after 14 to 16 days of incubation. First, their nuclei grow small and stain darkly, and then the small, not especially numerous D-granules appear (Villamil, 1942). Many dark islands are present by the eleventh day (Aron, 1931). As the exocrine tissue of the pancreas increases in amount, it insinuates itself between the dark islands, which then acquire a basal membrane that sets them off distinctly from the surrounding acini (Villamil, 1942).

The light islands develop by the multiplication of isolated cells or groups of cells that differentiate in the trabeculae or in the walls of tubules throughout the entire gland. These cells are recognizable by their increased amount of cytoplasm, which stains light blue with azan, and by their vesicular, chromatin-poor nuclei. After 12 days of incubation, B-granules, staining dull orange with azan, begin to appear in some of the cells (Villamil, 1942), and other cells start to degenerate (Potvin and Aron, 1927). Degenerative phenomena increase in intensity and reach a maximum at 13 to 15 days, then diminish. In some islands, all the cells degenerate; in others, only a few. At hatching, all the light islands are composed exclusively of B-cells (Villamil, 1942).

Explants of the 8- to 18-day chick embryo's pancreas, cultivated in plasma and embryonic extract, soon become surrounded by a zone of migrating cells consisting entirely or predominantly of connective tissue



elements (*Pinkus, 1930; Knake, 1935*). In cultures explanted in succession on the nineteenth to the twenty-first day of incubation, the epithelial (parenchymal) cells gradually increase in relative abundance and finally become the predominating type. This phenomenon has been related to the influx of yolk into the intestines during this period and the consequent functional activity of the pancreas (*Knake, 1935*). On the second or third day of culture, the epithelial cells migrate from the explant in the form of a coherent membrane. Parenchymal cells, containing zymogen granules, and the cells of the pancreatic ducts are both recognizable, and marked liquefaction of the plasma substrate occurs (*Pinkus, 1930*). Insular cells are not usually present; however, if plasma is replaced by agar, isolated groups of cells resembling islands of Langerhans may appear (*Black and Comolli, 1954*).







## CHAPTER SEVEN

# *The Respiratory System*

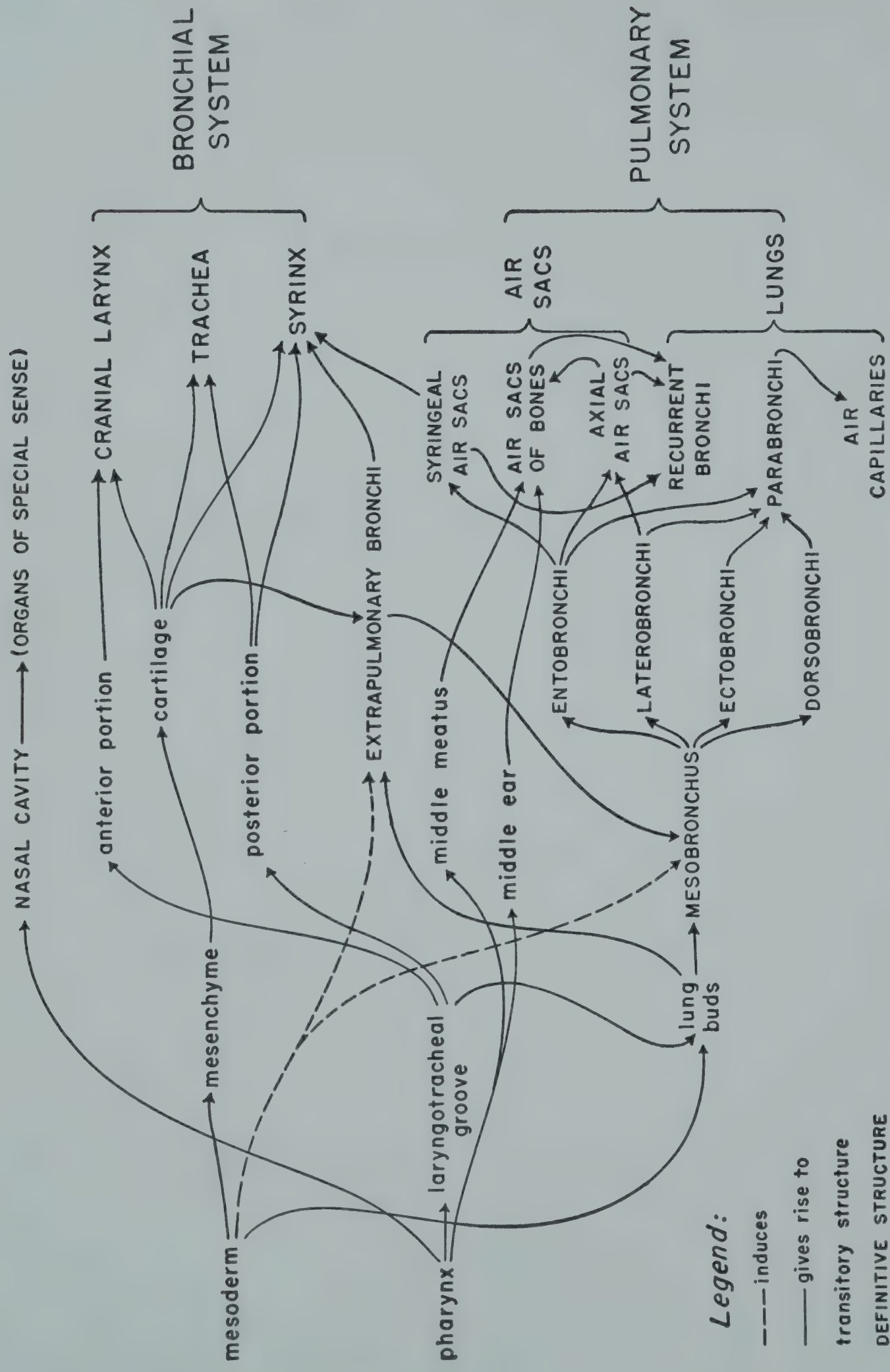
*It is for constant respiration—*

*Oxygenation of the blood;*

*The lungs start work at extrication*

*From shells . . . Each air sac is a bud.*







# THE RESPIRATORY SYSTEM

The respiratory system essentially includes those organs which are directly concerned with the intake of air, thus forming the passageway between the nostrils and the lungs. It also includes the lungs and air sacs which serve for oxidating the blood and rendering the body more buoyant in flight.

Upon entering the nostrils, inspired air is taken immediately into the nasal cavities. As it passes down the tract, it crosses the pharynx, which is also a food passageway. In the floor of the pharynx is the long, slitlike opening of the glottis. This opens to receive air into the cranial larynx, or superior larynx, which is actually the upper end of the long trachea, or windpipe. At the lower end of the trachea, just before the bifurcation into bronchi, is the syrinx, or true larynx. This highly specialized tracheal region is the organ of voice. The entire trachea is supported by cartilaginous rings, which are modified in both larynges to form special cartilages. The trachea ends by branching into right and left bronchi, each bronchus entering the ventral surface of the lungs. Once inside a lung, the main bronchus, or mesobronchus, gives off secondary bronchi of varying sizes, which communicate with air sacs, and from which tertiary bronchi radiate toward the surface of the lung. Each parabronchus or tertiary bronchus forms the center of the corresponding lobule of the lung.

The lungs are relatively small and occupy less than one sixth of the thoracic space. They are long, flattened, and oval, extending along each side of the spine from the first rib to the anterior end of the kidney and laterally to the juncture of the vertebral and sternal portions of the ribs. They are bright red, narrow anteriorly, and free on the dorsal surface. The lungs are composed of air vesicles which are minute but extremely numerous.

Birds are almost unique in the possession of air sacs, without which flight would be impossible. These sacs provide lightness and buoyancy for the body, and because of their dorsal position control the center of gravity. The air sacs receive air from the bronchi and occupy many bones of limbs and other parts of the skeleton.

The upper respiratory tract consists of the nasal chambers, pharynx, larynx, and trachea. The deeper and more posterior parts of the respiratory system are the bronchi, lungs, and air sacs. Embryologically, all portions of



the tract have their origin in the postbranchial region of the pharynx. The larynx and trachea start as a ventral groove, while the lungs form as paired outgrowths from the posterior lateral part of the embryonic pharyngeal tube.

### EARLY DEVELOPMENTAL POTENCIES

The usual method of determining the specific location of potential organ tissue in the early blastoderm is to transplant parts of the early embryo and see which organs develop therefrom in culture. It has been shown that mesectoderm tissue has the capacity to form so-called endodermal structures like the digestive tract and its derivatives. Portions of the respiratory tract develop from grafts of (1) mesectoderm of the entire area pellucida; (2) mesectoderm of the anterior streak level; (3) mesectoderm of levels posterior to the pit; and (4) mesectoderm of the streak, taken from hosts in the head-process stage, before the endoderm is established (*Hunt, 1937a*). Grafts of pellucid areas from chick blastoderm in the definitive streak stage and head-process stage contained potential pharynx, trachea, and lung tissue (*Willier and Rawles, 1931b*). Using chick blastoderms at stages from the head-process to 20 somites, Rudnick (1935) took grafts from the dorsal and ventral regions and found that respiratory tract epithelium was contained only in the ventral median region of the blastoderm.

The location of primordial lung tissue in the early chick blastoderm in the primitive streak stage was studied by Hunt (1932). Grafts were grown on the chorioallantois of 9-day chick embryos. Lung tissue developed in 8 per cent of the grafts taken as transverse cuts from the region anterior to the primitive pit and including the anterior half of Hensen's node. Grafts taken from the posterior half of the node and including area pellucida lateral to it, formed lung tissue in 50 per cent of the cases. The bronchial tubes of the lung are seen in grafts as epithelial-lined cavities which are surrounded by small knobs or plates of cartilage and condensed mesenchyme (*Hunt, 1932*).

The organization of potential areas of respiratory tract in the chick blastoderm is not specific and regular like the organization of thyroid, liver, or intestine. Instead, the differentiation is not precise and respiratory tubes are scattered in the blastoderm graft. Tubes of splanchnopleural origin appear to proliferate through the grafts without regional restraint or spatial organization. The grafted lung, for example, does not differentiate fully, either histologically or morphologically, unless the graft is taken after the rudimentary bud actually appears on the third day of incubation (*Rudnick, 1933*), and even then the number and pattern of secondary and tertiary branches is extremely variable (*Rudnick, 1952*).



The mesoderm seems to have a definite influence on the development of lung structure. It has been shown that the secondary and further branching of the endodermal tube of the lung bud and the differentiation of the lung parenchyma will not occur if the lung rudiment is divested of its outer mesenchymal coat. The mesoderm surrounding the bud forms early and the buds grow into this tissue. When mesoderm is removed, the epithelium is undamaged, yet further budding is inhibited. Thus the mesoderm at least plays a mechanical role and possibly induces development (*Rudnick, 1952*).

## THE UPPER RESPIRATORY TRACT

The parts of the upper respiratory tract are formed as single primordia, since their common anlage is the median, unpaired laryngotracheal groove. The nostrils, which are closely related to the sense organs, have a different origin and are described in detail in Chapter 5.

### The Nasal Cavities

In the bones of the head, there are certain expansions which, to all appearances, serve as air sacs. In a study of the pneumatization of the head of the fowl, *Bremer (1940b)* traced the development of large air cavities in the nasal region. The subocular sacs are large paired spaces between the nasal conchae. These sacs grow from the middle meatuses. The nasolacrimal passages are enormously dilated and connect laterally with the conjunctival sacs and medially with the floor of the nasal cavities. Both of these types of sacs develop without relation to the bone as simple ectodermal outgrowths but are ultimately enclosed by bone.

The subocular sacs begin on the seventh day of chick incubation as a flattened tubular outgrowth from the posterior end of the middle meatus, each sac extending forward and laterally, following the curve of the eye. The growing and spreading wall surrounds and fuses with arteries in its path so that arteries traverse the air sac (*Bremer, 1940b*).

The nasolacrimal passage, which drains tears from the conjunctival sac in mammals, is greatly expanded in birds. At 5 days, in the chick embryo, the ectoderm at the bottom of the lacrimal groove between the lateral nasal process and the maxillary process, grows inward as a broad, solid sheet. This loses connection with the surface epithelium by the seventh day. It grows to meet the floor of the nasal cavity, thus establishing the nasolacrimal duct. Two lateral branches grow out to the surface epithelium. A cavity is formed in the main cellular mass and extends medially until it opens into the nasal cavity.

The walls of the two sacs differ. The subocular sac, in the embryo, is lined by a simple layer of flattened cells, the connective tissue being very



slightly differentiated, while the lacrimal sac has columnar or pseudostratified epithelium and a dense outer coat. After hatching, the subocular sac is the thinner, containing cuboidal or columnar cells, while the lacrimal sac is pseudostratified and has goblet cells and small saccular mucous glands (*Bremer, 1940b*).

In addition to the sacs of the nasal cavity, spaces formed by the expansion of the wall of the tympanic cavity penetrate between the developing trabeculae of membrane bone or into the eroded primary marrow cavities of the cartilage to pneumatize the bones of the base of the skull.

Pneumatization of the head begins by the outgrowth of special tubular protrusions from the middle ear cavity. The bays grow into the mesenchyma and take in nerves and vessels which are in the path of growth. Rostrally, at about the fourteenth day of incubation, the dorsal part of the expanding wall meets the scattered trabeculae of the developing membrane bone of the supraorbital plates and folds around the small spicules forming bays between them. As more and more mesenchyma is converted into bone, the extension of the air sac follows.

Dorsally and caudally the tympanic expansion encounters the cartilaginous mass of the base of the skull. Here cortical trabeculae of bone are apposed to the surfaces of cartilage and the expanding cavity invades the cortical plates, and after the erosion of enclosed cartilage, it penetrates farther to invade the calcified matrix. Thus it fills the center of the bone with large air spaces, and the dorsorostral, dorsal, and caudal bones of the cranium are pneumatized.

To reach the more distal bones of the head and of the face, two special processes develop from the middle ear. They both arise before ossification occurs. One process is at the anterodorsal angle of the lateral wall of the tympanic cavity, and the second, appearing slightly later, comes from the anteroventral angle of the same wall. The dorsal sac appears at 9 days' incubation in the chick, coming in contact with the quadrate bone by flattening against the separate portions of the bone. The inner wall of the air sac bulges into the large, primary marrow spaces, pushing the marrow ahead and spreading as the erosion of cartilage proceeds. After the final union of the various bony elements of the quadrate, the whole bone is uniformly pneumatized. The air sac also extends to other bones with which the quadrate articulates, as in the case of the pterygoid bone. In this way many of the bones of the maxillary region are pneumatized.

The bones of the lower jaw are supplied by the second, more ventral outgrowth of the lateral wall of the tympanic cavity. The air space is enclosed by the cortex of the bone. In this case the air sac outgrowth must grow to a considerable length to meet the Meckel's cartilage and along its path it becomes almost degenerated at some points.



### The Pharynx

The embryonic pharynx is, in a sense, the alimentary canal of the head and anterior region. At its anterior extremity it is the preoral gut, extending in front of the oral plate. At this stage the embryo does not have a mouth but merely a depression in the ectoderm to mark the future site of the mouth. The depression deepens until it comes in contact with the endoderm of the pharynx. The thin layer of tissue between the stomodaeal ectoderm and the pharyngeal endoderm is the oral plate.

The branchial region of the pharynx covers the area where the visceral (or pharyngeal) pouches are evaginated as paired lateral expansions. The postbranchial portion of the pharynx is the site of evagination of the laryngotracheal groove. This groove is the first indication of the formation of the respiratory system. It appears as a midventral groove in the pharynx of the 3-day chick, and soon becomes separated by deepening and then closing at its dorsal margins. It remains attached to the pharynx only at the anterior (laryngeal) end.

The pharynx of the embryo is, in many ways, of greater significance than that of the adult, for it provides the origin of alimentary, respiratory, and glandular organs. In the adult it is an ill-defined cavity, and includes simply the part of the gut behind the mouth cavity but anterior to the esophagus. The morphogenesis of the pharynx is described in Chapter 6.

### The Trachea-Larynx-Glottis Complex

In its definitive state, the trachea-larynx-glottis complex resembles that of mammals in many respects. Points in which it differs include the absence of vocal folds in the larynx. These folds are associated with voice, which in birds is produced by the specialized syrinx. The thyroid cartilage is lacking among birds.

The cartilaginous ring structure of the trachea is quite typical of higher vertebrates. The rings are complete and are united by membranous ligaments. The rings are of alternating sizes, so that a large ring half overlaps a small ring and nearly touches the free edge of the next large ring. The next small ring likewise, nearly touches the edge of the previous small ring. The rings are thick around the middle and have thin anterior and posterior edges. The borders are usually joined by a band of connective tissue. Ossification occurs in the ventral part of the tracheal rings.

The origin of the trachea is in the laryngotracheal groove, which, when it appears in the 72-hour chick embryo, lies in the median ventral region of the postbranchial division of the early pharynx. Toward the end of the fourth day the trachea becomes differentiated from the posterior portion of the laryngotracheal groove. It may be definitely distinguished in an



embryo with 39 somites (approximately the 72-hour stage), and by the one hundredth hour it is well defined (*Locy and Larsell, 1916a*). This is clear in optical sections (see Fig. 217-B). It has been suggested that the trachea arises as an outgrowth of the posterior end of the laryngotracheal groove, but Rösler (1911) believed that it is probably formed from the rear portion of the groove by constriction of the pharynx. On the fourth day the walls of the lower end of the trachea and adjacent portion of the bronchi become thick and obliterate the lumen. This condition is very temporary.

The trachea elongates rapidly, keeping pace with the growth of the neck. On the sixth day the trachea is a long epithelial tube with thick walls at its posterior end. The cephalic end is expanded into a cavity which represents the larynx. The opening of the larynx-trachea into the embryonic pharynx is reduced to a slit which, at 6 days, is closed by a mass of epithelial cells. These are proliferated by the walls of the esophagus. The plug of epithelial cells is so thick as to obliterate the lumen of both the larynx and glottis of the 8-day chick embryo. The plug thins out, and, at 11 days, the cavity is open and the cells have formed a meshwork. During this time the glottis remains closed by fusion of epithelial cells. The time at which the glottis opens has not been observed.

The formation of cartilaginous rings has been observed in culture and suggests the manner in which differentiation occurs *in vivo*. Fore-gut complex taken from 7-day chick embryos and grown in culture produced well-formed tracheae and bronchi. After 7 or 8 days in culture isolated cartilaginous islands form outside the epithelium of these tubes, indicating the manner of normal growth (*Jong, 1938*).

### The Syrinx

The definitive syrinx is, in effect, the modified tracheobronchial junction. Both of these organs contribute to its formation. In general appearance, the vocal organ is membranaceous, with cartilaginous plates on the dorsal and ventral surfaces. The chamber, or tympanum, of the syrinx is laterally compressed. Ventrally, it more or less includes the area between the bifurcating bronchi. Bands of tissue which stretch between the bronchi serve to enclose a large air space between the bronchi and below the syrinx. The large ventral triangular cartilaginous plate is continuous with the dorsal plate by means of the semicartilaginous rod, the pessulus. The basal angles of the plates articulate with the extremities of the first bronchial semirings.

In addition to the supporting cartilaginous framework and membranes of the syrinx, the other structures involved in its composition are syringeal muscles and syringeal air sacs. The cartilage supports include, in addition to the dorsal and ventral plates and the rodlike pessulus, the last six tracheal rings and the first two bronchial rings. The membranes, which are



mostly stretched between the air sacs and give the syrinx its membranous appearance, include the *membranae externae*, *internae*, *tracheales*, *semilunaris*, and *bronchidesmus*. The muscles of the syrinx include the *sterno-tracheales* and the *tracheoclavicular*, which are related to the trachea as well. In addition, there are a dorsal and ventral pair of muscles which are true syringeal muscles and are relatively large. They are said to be derived from the *sternohyoid* group of muscles and to pass down the trachea to the syrinx, although in the chicken they reach only part of the trachea and do not extend as far down as the syrinx (Tymms, 1913). The respiratory air sacs associated with the syrinx are in intimate contact with one another and form a continuous envelope around the syrinx. These air sacs are derived from the third entobronchus (a branch of the main bronchial tube) of the lung. This particular sac expands into several sacs which help to surround the bifurcation of the trachea. The three principal sacs are a large ventral sac, a subpessular sac, and a dorsal sac which opens into dorso-lateral sacs.

**Embryonic Development.** As the trachea and bronchi develop, the surrounding mesenchyme begins to condense and concentrate around them on the third incubation day in the chicken (Tymms, 1913) and the seventh day in the sparrow, *Passer domesticus* (Wunderlich, 1886). Within the dense mesoblast, concentrations begin to assume a ringlike form. These are the rings that provide the cartilaginous framework of the trachea and bronchi. In the region of the bifurcation of the trachea they become specialized to form the supporting structure of the syrinx. The specialization begins on the ninth day in the fowl (Tymms, 1913) and the eighth day in the sparrow, *Passer domesticus* (Wunderlich, 1886). The pessulus forms between conjoining bronchi on the chick's eleventh incubation day (Fig. 210-A). At first it is bolt-shaped, with a rodlike connection between the dorsal and ventral triangular plates (Fig. 210-D). By the twelfth day, the fourth and fifth tracheobronchial rings appear and many tracheal rings have already appeared (Fig. 210-B).

The syrinx exhibits precocious sexual dimorphism after the eleventh day in the chick and the thirteenth day in the mallard duck (*Anas platyrhynchos*). It is the first organ after the gonads to undergo sexual differentiation. The syrinx of the male is much larger and more asymmetrical than that of the female (Wolff and Wolff, 1949b).

Because of the marked response of the syrinx to sex hormones, it is a useful biological indicator of the effects of castration or hormone treatment experiments (Wolff and Wolff, 1951b, 1952a, 1953; Wolff, Haffen, and Wolff, 1953). Castration produces the male-type syrinx. This type, therefore, is believed to be the neutral or asexual form of the organ, which is modified into the female type only after the appearance of sufficient female hormone (Wolff and Wolff, 1949b) (see Chapters 10 and 11).



In its gross developmental aspects the syrinx is generally asymmetrical. On the external surface of the organ the first sign of differentiation is the appearance of cartilaginous condensations and the rudiment of the mamillary protuberance (Fig. 211-A<sub>1</sub>). This appearance has been observed

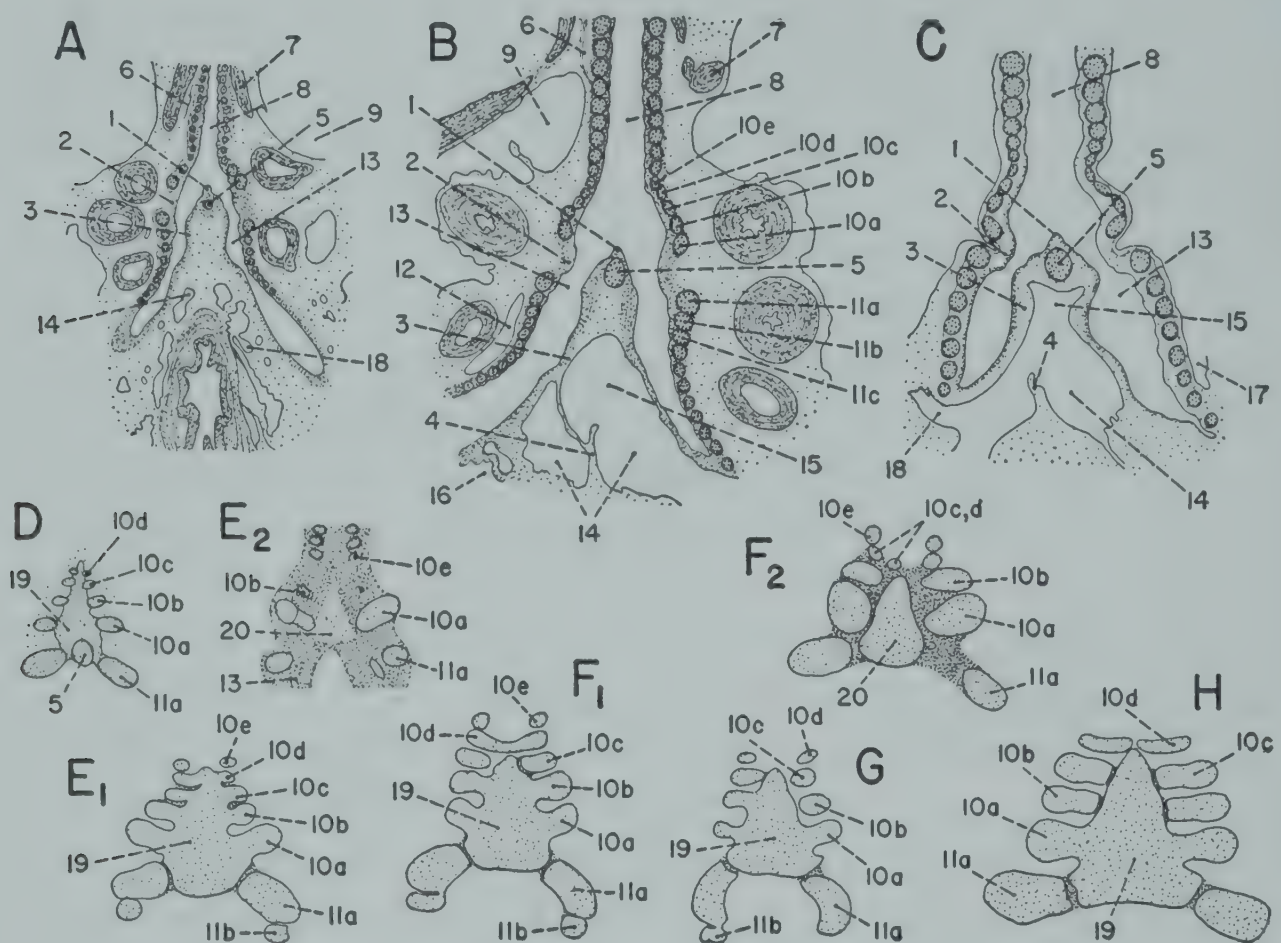


Fig. 210. Diagrammatic representations of stages in the embryonic development of the syrinx of the chicken (*Gallus gallus*). (Redrawn with modifications after Tymms, 1913.)

A, median frontal section through tracheobronchial region at 13 to 14 days' incubation ( $\times 12$ ); B, similar section showing condition at 15 days ( $\times 12$ ); C, similar section at hatching time ( $\times 12$ ); D, frontal section through ventral triangular plate of pessulus at 11 to 12 days' incubation ( $\times 8$ ); E<sub>1</sub>, ventral plate at 13 to 14 days' incubation ( $\times 8$ ); E<sub>2</sub>, dorsal plate at 13 to 14 days' incubation ( $\times 8$ ); F<sub>1</sub>, ventral plate at 15 days' incubation ( $\times 8$ ); F<sub>2</sub>, dorsal plate at 15 days' incubation ( $\times 8$ ); G, ventral plate at 16 days' incubation ( $\times 8$ ); H, ventral plate at hatching ( $\times 8$ ).

1, membrane semilunaris; 2, membrana externa; 3, membrana interna; 4, membrana bronchidesmus; 5, pessulus; 6, syringeal muscle; 7, tracheoclavicular muscle; 8, trachea; 9, cervical air sac; 10a, 10b, 10c, 10d, 10e, first through fifth tracheal rings, respectively; 11a, 11b, 11c, first, second, and third bronchial cartilaginous semirings, respectively; 12, dorsolateral air sac; 13, bronchus; 14, interbronchial air sac; 15, subpessular air sac; 16, lung tissue; 17, ventrolateral sac; 18, recurrent interclavicular branches; 19, ventral triangular plate; 20, dorsal triangular plate.

in the 4-day culture of the mallard embryo, *Anas platyrhynchos* (Wolff and Wolff, 1952b). The next occurrence is the fusion of the early cartilaginous arches with the tubercle (Fig. 211-A<sub>2</sub> and C), and the appearance of additional cartilaginous arches (Fig. 211-A<sub>3</sub>, B<sub>1</sub>, B<sub>2</sub>, and D). Variation in the fusion pattern occurs in culture (Fig. 211-B<sub>3</sub>). After 10 or 12 days in



culture, the outward appearance indicates a great increase in the cartilaginous framework (Fig. 211-E and F).

As the rings of the syrinx develop further, the supporting framework of the syrinx, the membranes, become differentiated from the surrounding tissue. The first to appear are the *membranae externae*, which are between the last tracheal ring and the first bronchial semiring (cf. Fig. 210-A and B). At this level, the wall of the trachea becomes deflected into the lumen and the tissue of the membrane becomes the very thin, oval-shaped membrane of the adult.

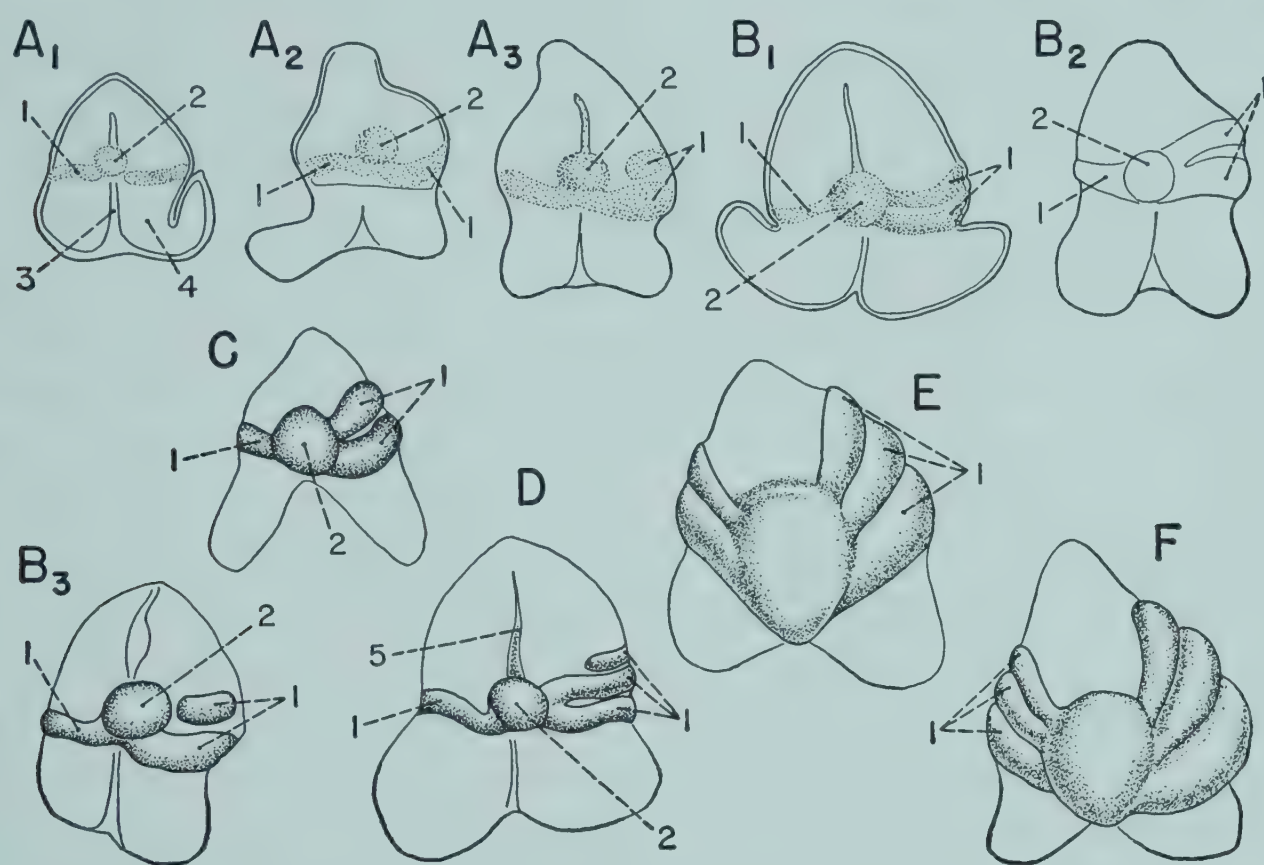


Fig. 211. Gross aspects in the development of the syrinx of the mallard duck (*Anas platyrhynchos*) in culture. (Redrawn with modifications after Wolff and Wolff, 1952b.)

A<sub>1</sub> to A<sub>3</sub>, beginning of first cartilages in syringes extirpated from embryo approximately 9 days old and cultured 4 to 5 days; B<sub>1</sub>, same syrinx after 5 days in culture; B<sub>2</sub> and B<sub>3</sub>, syringes of same age; C to F, approximately 9-day syringes cultured 7 days, 9 days, 10 days, and 12 days, respectively. A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, and B<sub>1</sub> are shown by transmitted light; the remaining, by reflected light. All  $\times 15$ .

1, cartilaginous arch; 2, mammillary protuberance; 3, pessulus; 4, chamber of right bronchus; 5, cartilaginous ridge.

After the chick's thirteenth incubation day, the interval between the last tracheal and first bronchial rings is greater, and thus the rudiments of the *membranae externae* are more marked. Near the end of the day the whole framework is of greater size and more definite outline. It has almost reached the cartilaginous stage. The boundaries of the pessulus are well-defined. Ventrally the last four tracheal rings are clearly involved in fusion with the sides and apex of the ventral triangular plate, while its basal angles articulate with the ends of the first bronchial semirings (cf. Fig. 210-E<sub>1</sub>). The dorsal triangular plate is appearing (Fig. 210-E<sub>2</sub>). The stem of the



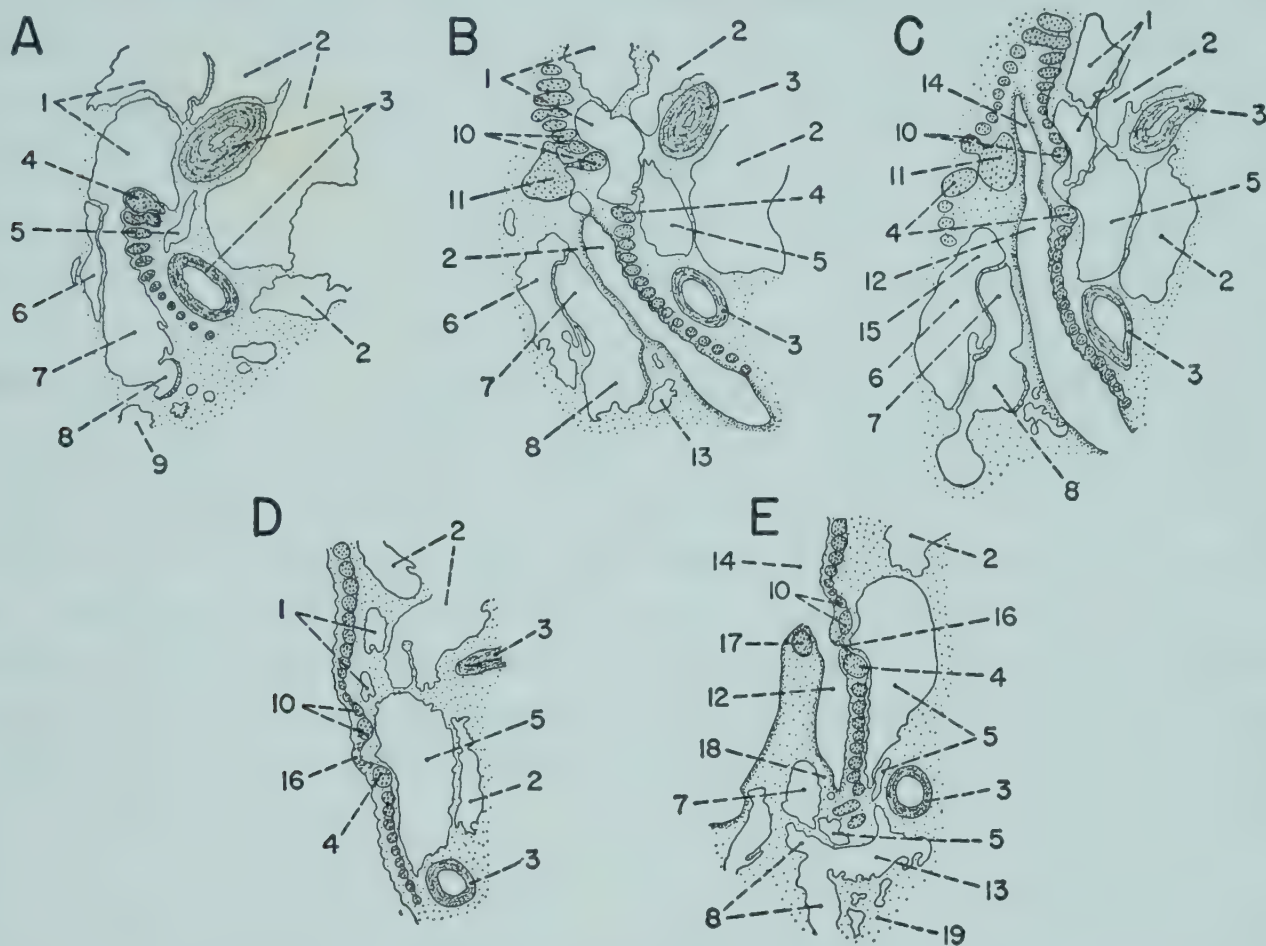
interclavicular sac and that of the anterior thoracic air sacs are formed of the bifurcations of the main stem of the third entobronchus of the lung. The stem of the interclavicular sac has divided by the fourteenth day into the large ventrolateral sac, the dorsal sac, and the subpessular sac. The syringeal sacs are not symmetrical, for the large subpessular sac is derived from the right side and is in contact with both *membranae internae* (Fig. 210-B). As a result, a three-layered band of tissue is gradually developed separating the air spaces of the right and left sides and tending to connect the two membranes obliquely. This band of tissue becomes the bronchidesmus, which in the adult is tough and fibrous (*Tymms, 1913*).

By the chick's fifteenth day the supporting framework is formed and all the syringeal membranes are indicated. The ventral ends of the third from the last tracheal ring are separated from the ventral plate (Fig. 210-F<sub>1</sub>). The tracheal rings and first bronchial semiring articulate with the dorsal plate (Fig. 210-F<sub>2</sub>). The muscles of the syrinx are developing and the air sacs begin to show intimate relationship with the syrinx as a whole. The surrounding mesoblast is less dense and begins to appear as a reticular meshwork, but the tissue around the lower tracheal rings is dense and made up of several layers of flattened cells. At this time also, the *membrana semilunaris* begins to form as a wedge-shaped fold which caps the ventral region of the pessular rod. The unattached edge projects slightly into the lumen of the tracheal extremity. The *membranae externae* are larger and are deflected into the lumen of the syrinx. At the same time the mesoblastic tissue lining the inner walls of the bronchi divide into two layers, thus forming the *membranae internae*. By this time the sternotracheal and tracheoclavicular muscles are well-marked and lie close to the ventral and lateral sides of the trachea. The latter muscles leave the walls of the trachea just above the region of the eleventh and twelfth from the last rings. Between these muscles and the trachea, the syringeal muscles are developing and extend down a short distance toward the trachea. They do not reach the syrinx and are not yet differentiated into a dorsal and ventral pair (*Tymms, 1913*.) Wunderlich (1886) noted that the syringeal muscles are fully differentiated on the eleventh incubation day in the sparrow (*Passer domesticus*) and on the twelfth day in the mallard duck (*Anas platyrhynchos*).

By the end of the chick's sixteenth day, only the extremities of the last tracheal ring and part of the penultimate ring are fused to the ventral plate (Fig. 210-G). The syringeal membranes, owing to the growth of the air sacs, take on their characteristic structure of three layers. The membranes are flanked on either side by an air cavity and function by vibration as the air spaces alternately expand and contract. In this final stage of embryonic development, the air sacs assume their definitive positions. The mesobronchus of the lung gives off its third entobronchus, which subdi-



vides into three branches. The first goes to the lung, where it bifurcates; one of these stems becomes the stem of the interclavicular syringeal air sac (Fig. 212-A). The other bifurcation expands into the large anterior thoracic air sac. The stem of the interclavicular sac, after emerging from the lung, passes close to the ventromedial region of the bronchus and begins to expand into several large air sacs, all connected with the syrinx.



**Fig. 212.** Longitudinal sections showing the relationship of air sacs in the tracheo-bronchial region of the fowl (*Gallus gallus*) at 16 days' incubation. (Redrawn with modifications after Tymms, 1913.)

A, section through level of interclavicular sac; B, through level of interbronchial sac; C, section showing relation of interbronchial and lateral sacs to membranae externae and internae; D, through dorsolateral sac; E, through main recurrent interclavicular sac. All  $\times 30$ .

1, ventrolateral sac; 2, cervical air sac; 3, large blood vessel; 4, first bronchial semiring; 5, dorsolateral sac; 6, right interbronchial sac; 7, left interbronchial sac; 8, interclavicular air sac; 9, anterior thoracic sac; 10, lower tracheal cartilaginous rings; 11, ventral triangular plate; 12, bronchus; 13, small recurrent interclavicular branch; 14, trachea; 15, subpessular sac; 16, membrana externa; 17, pessulus; 18, membrana interna; 19, lung tissue.

These include the ventrolateral sac; the subpessular sac, which extends upward between the bronchi to the pessulus (Fig. 212-B); and the large dorsal sac, which gives off three divisions, cranial, caudal, and lateral (Fig. 212-C). Of the last three divisions, the first occupies the dorsolateral region (Fig. 212-D) of the syrinx, the second passes to the lateral position, the third and largest branch gives off diverticula to the lung. These diverticula at the third branch form the recurrent branch of the interclavicu-



lar sac (Fig. 212-E). Thus all surfaces of the syrinx are enclosed by air sacs.

From the seventeenth incubation day until hatching, the development of the syrinx is confined largely to growth. Histologically, the principal change is the deposition of the cartilaginous matrix. Also, the rings undergo some further modification. The next to the last ring ceases to be attached to the ventral triangular plate, so that only the last ring is fused to the plate (cf. Fig. 210-H). Dorsally, the fifth from the last ring has downturned ends which are bound to the apex of the plate by tissue which becomes fibrous. The rings of the *membranae tracheales* become flattened. The *membranae internae* and *externae* have become thinner. The syringeal muscles are more strongly developed but extend only part way down the trachea.

**Postembryonic Changes.** There is considerable development of the vocal organ after hatching. Most of the changes are histological. The tracheobronchial junction is converted into a true membranaceous structure, largely because the last five tracheal rings (which are embedded in the organ) become extremely thin vestiges. The *membranae externae* become the most distinctive *membranae*. Thin, strong, and oval, they are stretched between the last tracheal ring and the well-marked first bronchial semiring. The *bronchidesmus* becomes fibrous. The dorsal and ventral plates thicken (*Tymms, 1913*).

## THE LOWER RESPIRATORY TRACT

The more posterior and deeper parts of the respiratory system are derived from paired endodermal outpocketings of the embryonic fore-gut. The principal organs, of course, are the lungs, connected with the trachea by means of the bronchi. The primitive bronchi consist of two parts, one leading to the lung (extrapulmonary bronchus) and the other extending into the lung (mesobronchus). The mesobronchus gives rise to the air passages of the lung and to the air sacs, which extend into various parts of the body.

### The Lungs

There are several important anatomical features which distinguish the avian lung from the mammalian and other vertebrate types. The development of recurrent bronchi from the air sacs is unique, as is also the establishment of labyrinthine communications between all parts of the bronchial tree. In other vertebrates, the bronchial branches end in cul-de-sacs; but the fact that the branches anastomose in the bird's lung means that facilities for air exchange are extremely efficient. The absence of alveoli permits inspired air to travel unimpeded through the minutest air passages.



The air sacs receive their supply through direct orifices during inspiration; during expiration, the air passes from these reservoirs into the lung by way of the recurrent bronchi.

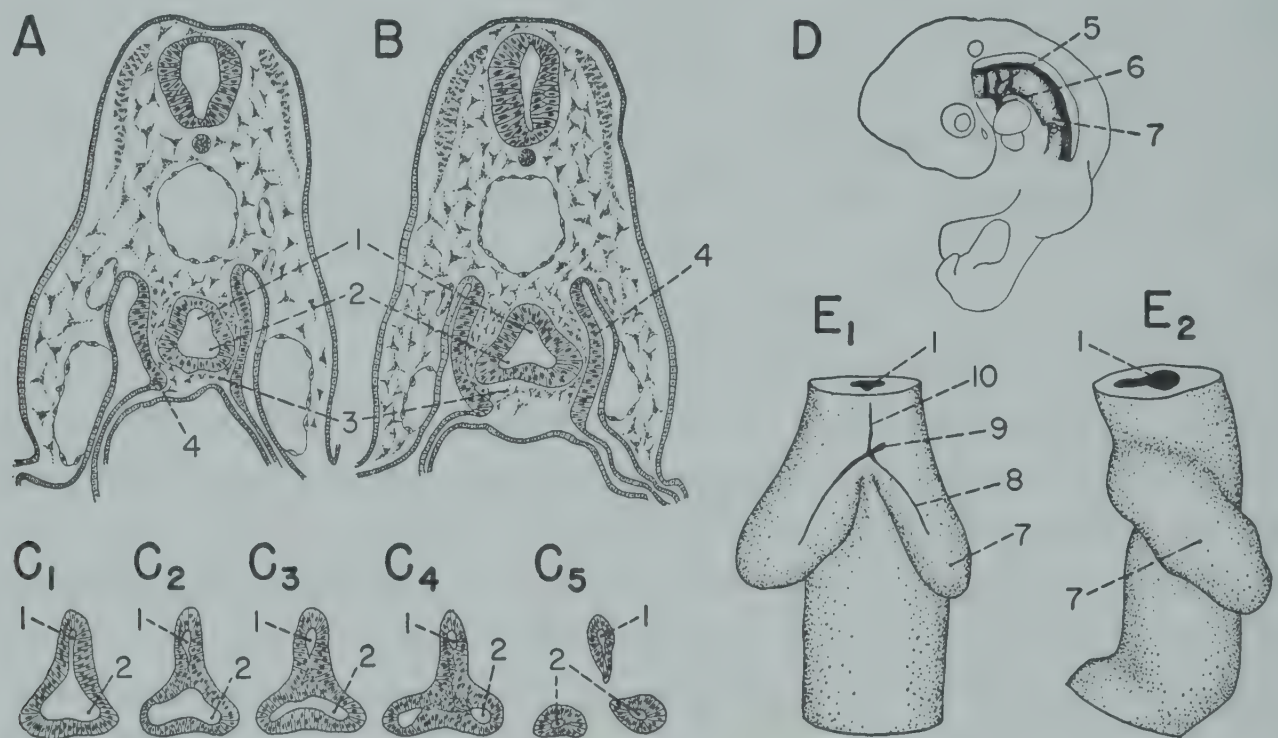
The lungs first arise as diverticula from the fore-gut at a level posterior to the fourth visceral pouch and just ventral to the esophagus. Actually the paired diverticula arise from the caudally growing end of the laryngotracheal groove. The tips of the diverticula become the lungs while the proximal portions are destined to form bronchial tubes. The paired endodermal tubes grow into the surrounding mesenchymal mass and the mesoblast arranges itself around the buds so that two lobes of tissue are formed. Expansion continues within the pleural cavities. The lungs are attached to the laryngotracheal groove by their ventral surface, which is a plane surface. By continued growth the lungs arch dorsally and anteriorly, and an anterior lung lobe soon forms projecting in front of the mesobronchus. Thus part of the mesobronchus is outside of the lung, while the rest eventually ramifies throughout the lung tissue. The gross picture of the lungs is that of a bright red, spongy mass penetrated by air tubes of varying sizes and innumerable capillaries.

**Pulmonary Morphogenesis.** The lungs are paired from the very beginning. They first appear early during the third day of the chick's incubation (*Locy and Larsell, 1916a*). At this time the lung primordia are no more than a ridgelike formation on each side of the ventral surface of the primitive fore-gut, or pharynx, just caudad to the fourth pharyngeal pouch and below the broader branchial region of the fore-gut. In cross section of the 50-hour incubated chick (Fig. 213-A) each ridge can be seen as an evagination of endoderm pushing into the surrounding mesenchyme. In the next 2 hours (Fig. 213-B) the pharynx appears more triangular, because of the evagination of the lung buds. A well-defined mesothelium borders the mesenchyme around the lung buds at this stage. The surrounding mesoderm is also a part of the rudimentary lung, developing at the same pace and providing material for blood vessels, lymph spaces, muscles, connective tissue, and other elements associated with lung development. The endoderm buds to provide the lining membrane of the bronchial tree. Anterior to the lung buds, the floor of the pharynx is narrowed to form the laryngotracheal groove, and this area is constructed from the compressed tube above. The cavity of the pharynx is narrowed above the lung evaginations (Fig. 213-C<sub>1</sub>). In quick succession the stages depicted in Fig. 213-C<sub>2</sub> to C<sub>5</sub> follow after the fifty-fifth hour. The endodermal lung pouches elongate and grow in a caudolateral direction, and their divergent distal ends become separated from the esophagus before the sixtieth hour.

At the end of the fourth day (*Locy and Larsell, 1916a*) or at 41 somites (*Rösler, 1911*), the lungs are small, smooth pouches extending caudad along each side of the esophagus (Fig. 213-D). In ventral view (Fig.



213- $E_1$ ) and lateral view (Fig. 213- $E_2$ ) it is impossible to see the true direction of the anlagen, which extend caudad, dorsally and laterally. The right mesobronchus is 300 microns long, the left is 230 microns, and the trachea is 120 microns long (Rösler, 1911). Locy and Larsell (1916a) claim that differentiation of the trachea is not yet evident from the surface of the pharynx. While the distal ends of the lungs diverge, the bases of the evaginations protrude from the wall of the esophagus in the midventral



**Fig. 213.** Development of the lung in the 2- to 4-day chick embryo (Redrawn with modifications after Locy and Larsell, 1916a.)

A, transverse section through a 51-hour embryo at level of pharynx and lung pouches ( $\times 30$ ); B, similar section through an embryo approximately 1 hour older ( $\times 30$ ); C<sub>1</sub> to C<sub>5</sub>, successive stages in separation of distal end of lung pouches from esophagus occurring between fifty-fifth and fifty-sixth hour ( $\times 33$ ); D, left lung pouch and aortic arches of 96-hour embryo ( $\times 4$ ); E<sub>1</sub>, ventral, and E<sub>2</sub>, lateral surface views of lungs at the end of 4 days' incubation ( $\times 40$ ).

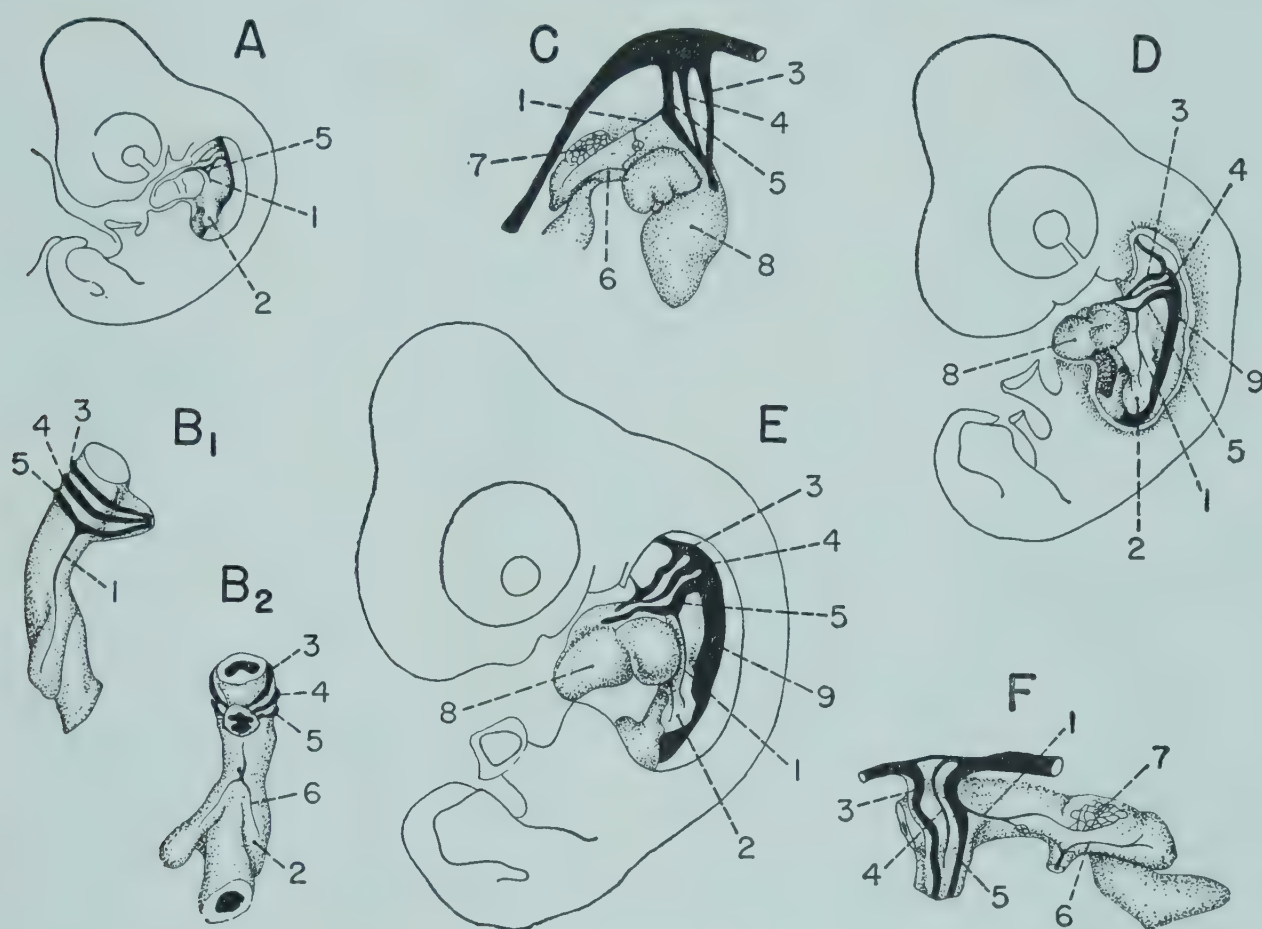
1, lumen of pharynx; 2, lung pouch; 3, mesenchyme; 4, mesothelium; 5, third aortic arch; 6, sixth aortic arch; 7, left lung; 8, pulmonary vein; 9, vein to left atrium of heart; 10, view from laryngotracheal groove.

line and unite with the laryngotracheal groove just anterior to the lungs. Vascularization, at least of the surface of the lung pouches, is already evident. The blood vessels meet in the median ventral plane and at the point where the lungs join the pharynx; these are the pulmonary veins (see Chapter 8). At the end of the fourth day the distal end of the mesobronchus is enlarged to form the abdominal air sac. At the proximal end of the lung, on each side, part of the bronchus lies between the bases of the lungs; this is the primordium of the extrapulmonary bronchus. The extrapulmonary bronchi eventually join the trachea.

On the fifth day, the lung begins to show surface irregularities. The lung pouch has assumed a position dorsal to the aorta (Fig. 214-A). At its



distal end, a protuberance (Fig. 214-B<sub>1</sub>) represents the beginning of a lobe which will contain the mesobronchus. At this time the pulmonary artery is established as a spur from the sixth aortic arch (Fig. 214-A). In ventral view, the right lung forms a greater angle with the esophagus than the left, producing slight asymmetry (Fig. 214-B<sub>2</sub>), which is due mainly to pressure from the enlarging stomach. By the end of the fifth day a network of blood vessels lies near the dorsal anterior surface of the lungs



**Fig. 214.** Stages in the external development of the chick embryo's lung from the fourth to the seventh day of incubation. (Redrawn with modifications after Locy and Larsell, 1916a.)

A, lung and adjacent structures in 4.5-day (114-hour) embryo ( $\times 3$ ); B<sub>1</sub>, lateral, and B<sub>2</sub>, ventral views of 4.5-day embryo ( $\times 11$ ); C, lung and related structures at the end of the fifth day of incubation ( $\times 9$ ); D, left side of 5.5-day embryo ( $\times 3$ ); E, left side of embryo on last half of sixth day ( $\times 3$ ); F, lung and related structures early on seventh day ( $\times 7.5$ ).

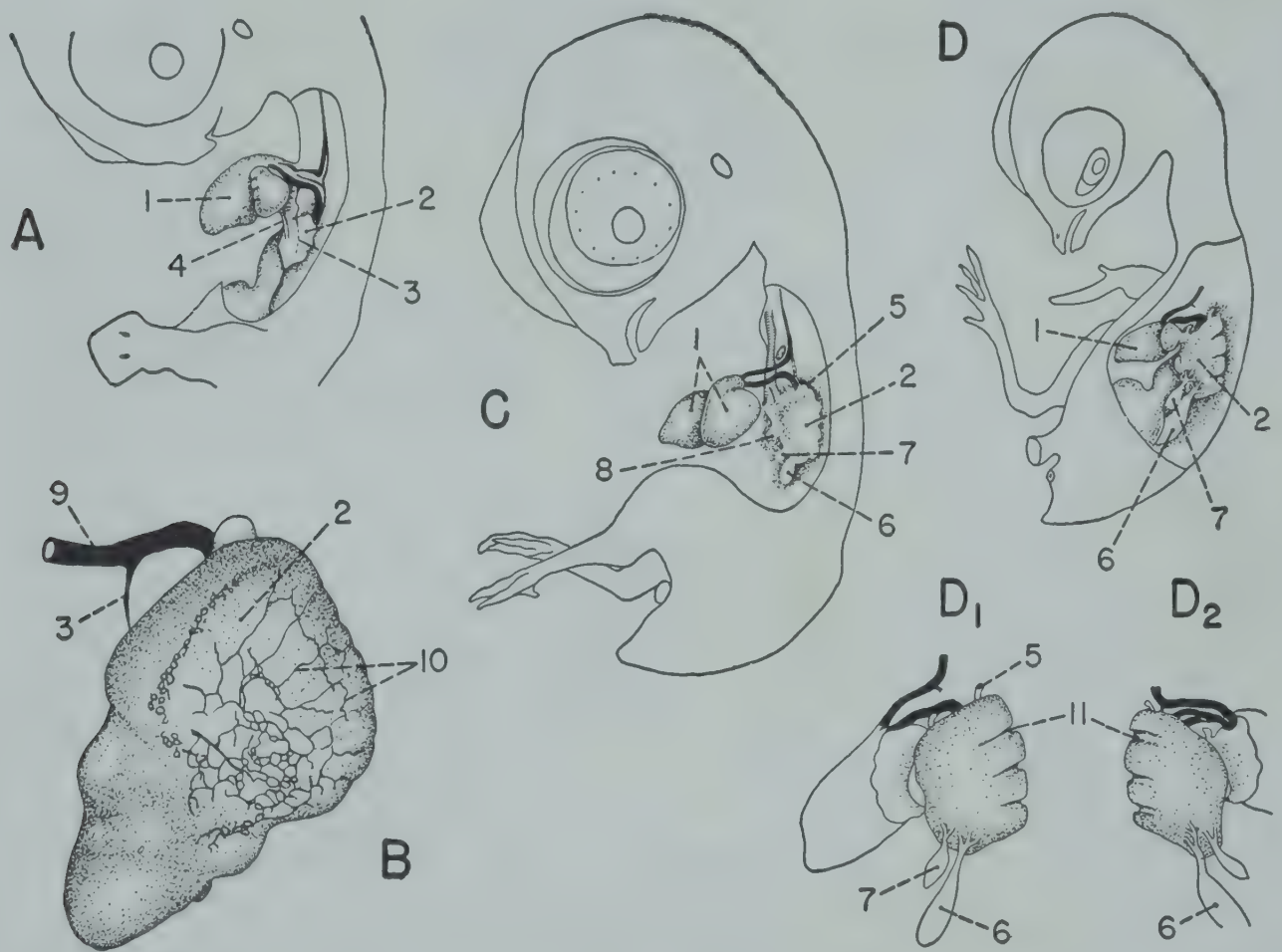
1, pulmonary artery; 2, left lung; 3, third aortic arch; 4, fourth aortic arch; 5, sixth aortic arch; 6, pulmonary vein; 7, blood vessel plexus; 8, heart; 9, aorta.

(Fig. 214-C). A branch from the pulmonary artery extends to the trachea. At 5.5 days (Fig. 214-D) and 6.5 days (Fig. 214-E), the dorsal (cephalic) part of the lung increases its protrusion and the hooklike caudal process also enlarges. The superficial blood vessels can be seen on the seventh day, as shown in Fig. 214-F. At this stage, the trunk of the pulmonary vein forms a juncture with a vein which comes from the laryngotracheal groove. A short branch from the pulmonary artery passes through a capillary network and joins the laryngotracheal vein. On the sixth day, external



changes are not conspicuous, but the first branches of the bronchial tree are formed internally.

The following day sees a change in shape of the lung to a rectangular organ with protuberances at both the cephalic end and caudal extremities (Fig. 215-A). Two air sacs project from the anterior margin and three from the ventral margin of the lung (Fig. 215-B). The blood supply is concentrated in the dorsal region of the lung (Fig. 215-C). The tenth day is marked by conspicuous indentations of the dorsolateral border of the



**Fig. 215.** Stages in the development of the lung and related structures in the chick embryo from the seventh to the thirteenth day of incubation. (Redrawn with modifications after Locy and Larsell, 1916a.)

A, lung region of embryo at end of seventh day ( $\times 4$ ); B, capillary network on dorsolateral surface of left lung at end of eighth day of incubation ( $\times 35$ ); C, lung region of 9-day embryo ( $\times 3$ ); D, 13-day embryo ( $\times 1.5$ ); D<sub>1</sub> and D<sub>2</sub>, gross aspects of lungs of 13-day chick embryo ( $\times 3$ ).

1, heart; 2, lung; 3, pulmonary artery; 4, pulmonary vein; 5, cervical air sac; 6, abdominal air sac; 7, posterior intermediate sac; 8, anterior intermediate sac; 9, aorta; 10, capillary; 11, indentation due to rib pressure.

lungs caused by pressure of the ribs against the lungs. Generally there are four deep grooves (Fig. 215-C and D). At this time also, the lungs move to a more dorsal position, the dorsal margin coming to lie along the vertebral column. The recurrent bronchi are formed on the ninth day as buds from the proximal ends of the abdominal and the posterior intermediate air sacs, and later, from other air sacs. While the air sacs are developing, the lungs increase in size, and in a few days the recurrent bronchi anastomose with parabronchi.



The principal changes in the lung in the next few days involve the air sacs. By the thirteenth day, the lungs are quite lateral in position in the thoracic cavity, the indentations are deep (Fig. 215-*D*<sub>1</sub> and *D*<sub>2</sub>), and five air sacs with stems of recurrent bronchi are present. The development of the lungs (for weight, see Appendix, Table V) has reached the stage which exists at hatching time. The weight of the lungs near hatching time is given in the accompanying table.

Age of Chick Embryo	Temperature of		Investigator
	Incubation (centigrade)	Weight of Lung (grams)	
Hatching		0.25	Latimer (1924)
Hatching	36.0°	0.29	Romanoff ( <i>unpublished</i> )
Hatching	37.5°	0.27	Romanoff ( <i>unpublished</i> )
Hatching	38.5°	0.25	Romanoff ( <i>unpublished</i> )
Second day after hatching		0.33	Latimer (1924)

**Vascularization of the Lungs.** The principal vessels of the lung are the pulmonary artery and the pulmonary vein. The intermediate capillary network is also a vital part of the vascular mechanism.

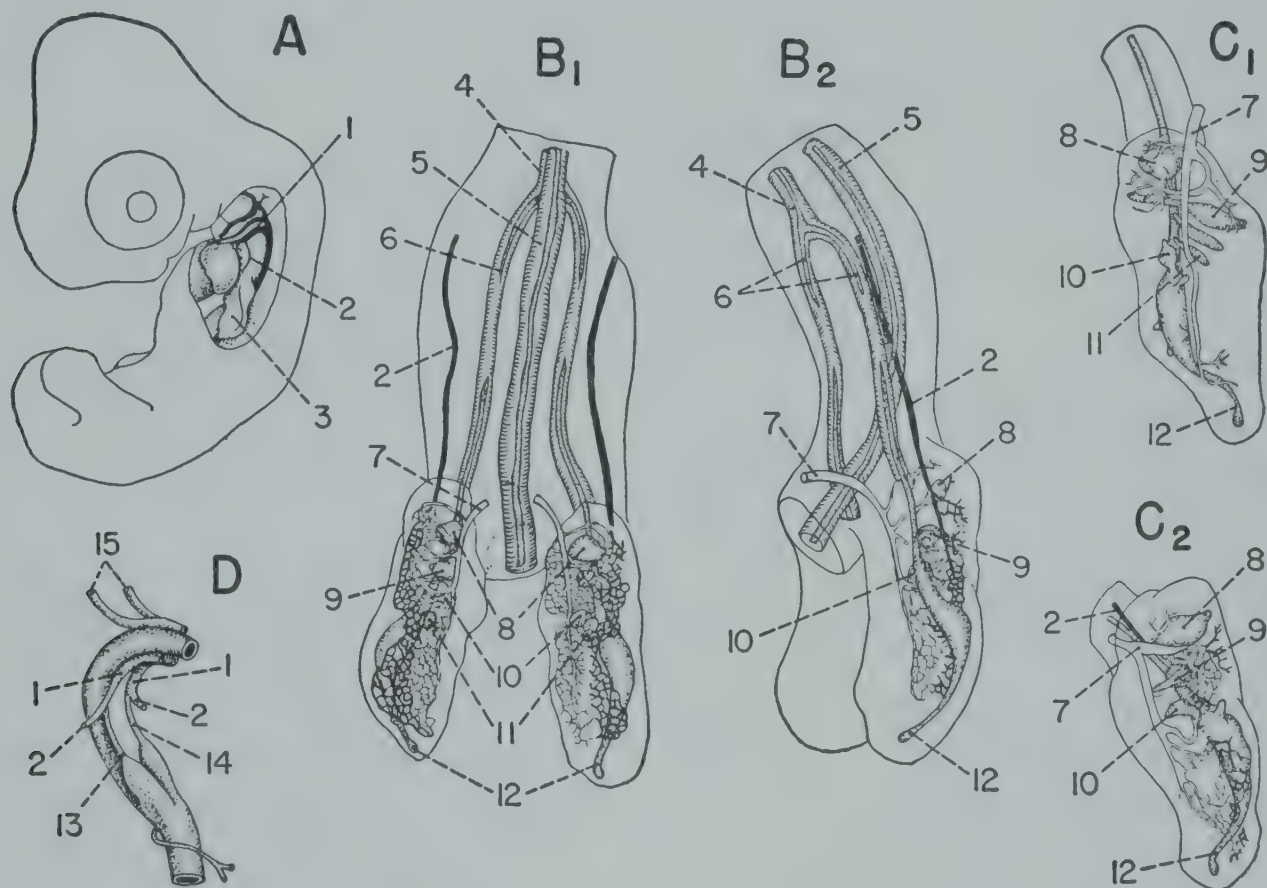
Before the lung is vascularized, it is surrounded by vascular spaces. These can be seen in the mesenchyma of the lung primordium of the 52-hour chick embryo. At first these sinusoidal spaces are small, but they gradually come together and form an incipient network of capillary-like canals. By the eighty-second hour, the canals occupy the area of the lung in which the pulmonary artery will arise. These vascular changes mentioned occur long before the formation of the sixth aortic arch.

The pulmonary vein is the first vessel to appear. In the ventral aspect of the 4-day chick embryo, there is a definite blood vessel on the ventral surface of each lung, and these two vessels unite to form a median trunk vessel (cf. Fig. 213-*E*<sub>1</sub>). Anterior to this is a median vessel along the laryngotracheal groove. The pulmonary artery is formed between the fourth and fifth days from two parts, the proximal end coming from the sixth aortic arch, and the distal end beginning in the lung wall and growing to meet the sprout from the aorta. At 4.5 days the completed pulmonary artery can be seen (Fig. 216-*A*). Both ends are well developed, but in the middle the vessel is threadlike. The distal division from the lung is shorter than the proximal division from the sixth arch (*Locy and Larsell, 1916a*). At the end of the fifth day, the pulmonary artery runs nearly parallel to the extrapulmonary bronchus and enters the lung dorsal to the bronchus. The pulmonary vein runs through the ventral region below the central lung tube. The artery branches and divides into capillary networks around the entobronchi (Fig. 216-*B*<sub>1</sub> and *B*<sub>2</sub>). The capillary network of the



dorsal side surrounds the lung tube and its outgrowths, passes ventrally, and meets the capillary network of the pulmonary vein.

The branching of the blood vessels within the lungs increases rapidly toward the end of the fifth day. Figure 216-C<sub>1</sub> shows the chief branches of the pulmonary vein, while Fig. 216-C<sub>2</sub> shows the veins, arteries, and a simplified diagram of the capillaries.



**Fig. 216.** Early stages in the development of the air sacs and the vascular supply of the lungs in the chick embryo. (Redrawn with modifications after Locy and Larsell, 1916b.)

A, embryo at 4.5 days ( $\times 4$ ); B<sub>1</sub>, dorsal and B<sub>2</sub>, lateral aspects of lungs of 6-day embryo, showing arteries, veins, and capillary network ( $\times 18$ ); C<sub>1</sub>, pulmonary vein and left lung of 6-day embryo ( $\times 18$ ); C<sub>2</sub>, pulmonary vein and artery of right lung at same age ( $\times 18$ ); D, pulmonary artery of newly hatched fowl ( $\times 18$ ).

1, sixth aortic arch; 2, pulmonary artery; 3, lung; 4, trachea; 5, esophagus; 6, bronchus; 7, pulmonary vein; 8, entobronchus 1; 9, entobronchus 2; 10, entobronchus 3; 11, entobronchus 4; 12, abdominal air sac; 13, right ductus Botalli; 14, left ductus Botalli; 15, third aortic arch.

In the 8-day embryo, the capillary network occupies the anterodorsal region of the lung. By the tenth incubation day, the entire laterodorsal surface is covered by a network of vessels.

The lung of the chick does not begin to function until shortly before hatching. Thus the pulmonary arteries remain relatively small, and blood from the right ventricle passes through both the left and right divisions of the sixth aortic arch to enter the aorta. At hatching, the pulmonary arteries are larger, and the proximal portions of the sixth aortic arches (the ducti arteriosi or ducts of Botalli) undergo an obvious constriction. In the



adult system the ductus Botalli is lost (see Chapter 8). The condition at hatching is shown in Fig. 216-D.

### The Bronchial Tree

The bronchial system within the lung comprises a unit quite distinct from the air sacs or the extrapulmonary bronchial system. The unique feature of the avian bronchial tree is the absence of blind endings of the bronchial twigs, and the formation, instead, of an anastomosing network. Thus the term bronchial tree refers to the central trunk and the larger branches with their subdivisions (*Locy and Larsell, 1916b*).

#### Gross Morphology

In the definitive state, the bronchial system includes the paired extrapulmonary tubes which begin anteriorly at the syrinx and conduct air from the trachea into the lungs. These tubes are supported by semirings of cartilage, incomplete ventrally, and are lined by a mucous, ciliated membrane similar to that of the trachea. In the bronchi, a layer of circular involuntary muscle forms of muscularis mucosae external to the mucosal lining.

Within the lung, the main bronchus (mesobronchus) develops an ampullary dilation, the vestibulum, which gives off secondary bronchi (ecto-, endo-, and laterobronchi) of different sizes. These communicate directly or indirectly with the air sacs and also give off tertiary bronchi which pass into the lung and radiate in a pennate manner toward the surface of the lung, where they end blindly. The walls of the tertiary bronchi have numerous small openings leading to minute canals, which are dilated like the air vesicles of the mammalian lung and perform the same function. These dilated canals are separated from each other by thin septa containing a close-meshed network of capillaries that connect the pulmonary artery and pulmonary veins. Each tertiary bronchus forms the center of a lung lobule.

It has been demonstrated by the use of hypotonic solutions that a lining of connective tissue is present in all walls of the system of air tubes. In fact, dying provides evidence that air in all parts of the lung is enclosed by an epithelial layer (*Bremer, 1939*).

**Early Development.** The earliest indication of the bronchial tree is seen in the hollow buds that form from the endoderm lining the lung pouches on the chick's third day of incubation (Fig. 217-A<sub>1</sub> and A<sub>2</sub>). At 96 hours the rudiment of the bronchial tree is a simple cylindrical tube of endoderm which extends into the surrounding mesenchymal part of the lung primordium. At the same time, the trachea has begun to differentiate from the laryngotracheal groove. At the proximal end of the lung bud a small part lies outside the mesenchymal swelling, and this part forms the



primordium of the extrapulmonary bronchus. At this time the trachea is formed and the narrow bronchi connect with it (Fig. 217-B).

During the latter half of the fifth day, a spindle-shaped expansion forms within the lumen of the mesobronchus (Fig. 217-C). This expansion has been interpreted by Locy and Larsell (1916a) as an embryonic vestibulum that does not correspond to the vestibulum of the adult lung. It is from this expanded region that the ectobronchi arise. The expansion also serves

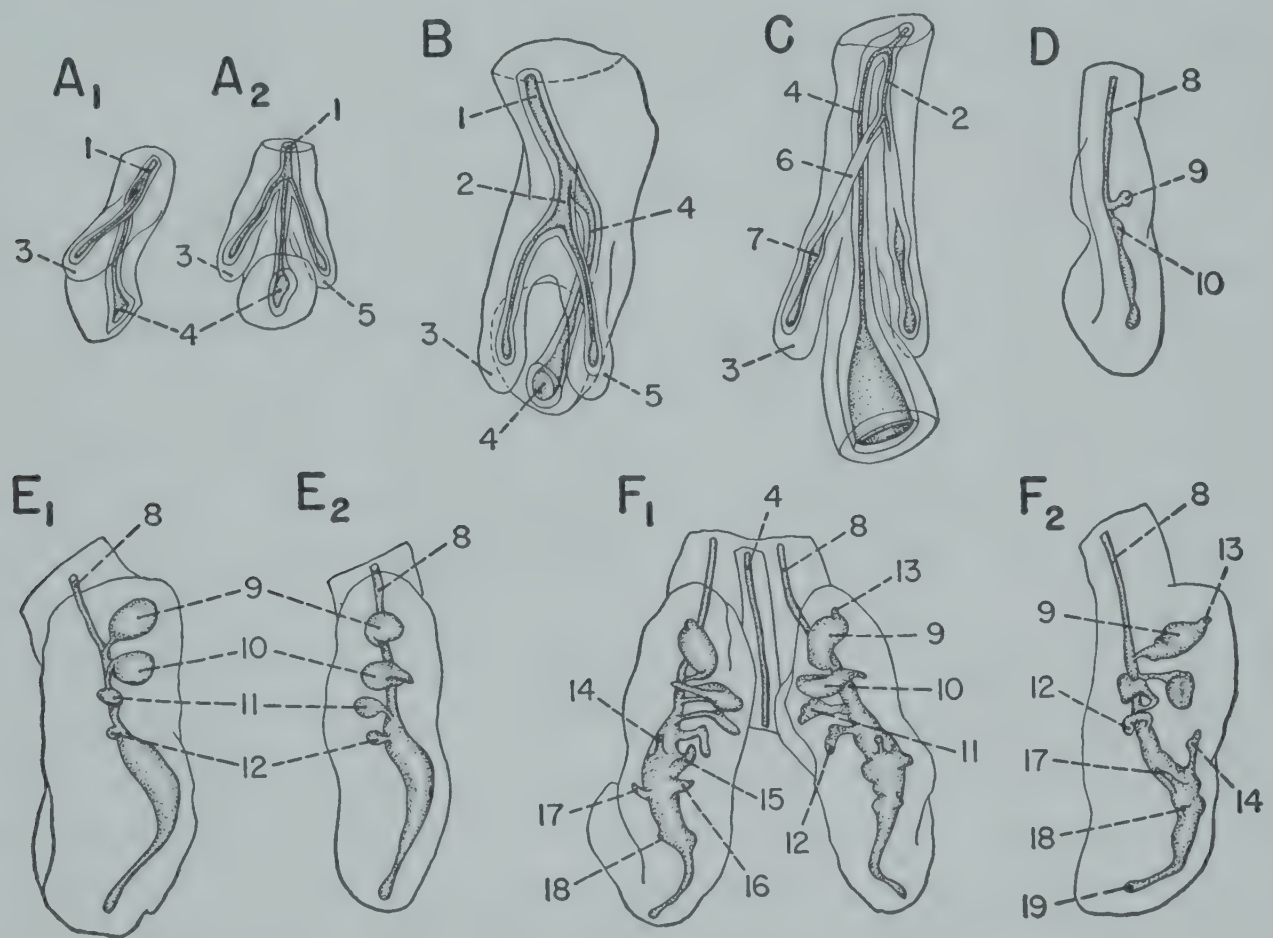


Fig. 217. Development of the bronchial tree in the chick embryo's lung from the fourth day to the end of the sixth day of incubation. (Redrawn with modifications after Locy and Larsell, 1916a.)

A<sub>1</sub>, lateral and A<sub>2</sub>, ventral views of central lung tube of a 96-hour embryo ( $\times 12$ ); B, ventrolateral view of lungs in a 100-hour embryo ( $\times 15$ ); C, lungs at 5 days ( $\times 15$ ); D, lung at 5.5 days ( $\times 15$ ); E<sub>1</sub>, lateral view and E<sub>2</sub>, dorsolateral view of a 6-day lung system ( $\times 17$ ); F<sub>1</sub>, dorsal view and F<sub>2</sub>, lateral view of a 6.25-day embryo ( $\times 17$ ).

1, pharynx; 2, trachea; 3, right lung; 4, esophagus; 5, left lung; 6, occluded portion of bronchus; 7, embryonic vestibulum of central lung tube; 8, bronchus; 9, entobronchus 1; 10, entobronchus 2; 11, entobronchus 3; 12, entobronchus 4; 13, cervical air sac; 14 to 16, ectobronchi 1 to 3; 17, laterobronchus 2; 18, laterobronchus 3; 19, abdominal air sac.

to separate the lung into an interior, a middle, and a posterior part (Selenka, 1866).

The sixth day marks the formation of buds from the primary lung tree. These buds represent the beginnings of the secondary branches of the bronchial tree. The first bud is formed from the internal wall of the intrapulmonary bronchus (Fig. 217-D) and is the primordium of the first entobronchus. Behind this is the smaller bud of the second entobronchus. Immediately afterward, a third and fourth bud initiate the corresponding



entobronchi (Fig. 217- $E_1$  and  $E_2$ ). They remain connected to the bronchus by slender stalks. They then curve around the bronchus so as to occupy the ventral face of the lung (*Juillet, 1912*).

The bronchial branches have been designated by various systems of nomenclature. According to Sappey (1846), they are either diaphragmatic branches (directed ventrally) or costal branches (extending dorsally). More frequently, they are divided into primary, secondary, and tertiary bronchi.

The primary bronchus is the mesobronchus, often called the central lung tube. The secondary bronchi can be divided into four groups. The five large divergent bronchi are the diaphragmatics of Sappey, now referred to as the entobronchi; the eight internal bronchi are the costals of Sappey, now called the ectobronchi; the six external bronchi are called laterobronchi; and the posterior or dorsal bronchi are called dorsobronchi by Locy and Larsell (1916a). The tertiary bronchi are the terminal branches of the subdivisions of the secondary bronchi and are generally called the parabronchi or air pipes. In addition to these tubes, there are recurrent bronchi, which come from the air sacs, and the air capillaries, which are radially arranged around the parabronchi.

The initial formation of the four entobronchi has already been described. On the seventh day the primordia of the ectobronchi appear as a series of buds from the wall of the embryonic vestibulum, below the level at which the entobronchi formed (Fig. 217- $E_2$ ). Six buds are usually found in the chick, but as many as ten may be present (*Locy and Larsell, 1916a*). The first develops more rapidly than the others and is soon a long curving tube projecting forward and laterally (Fig. 217- $F_1$  and  $F_2$ ).

Near the end of the seventh day, a number of incipient branches, the laterobronchi, spring from the central lung tube. In the adult they are small but are coordinate with the ento- and ectobronchi. The first three are shown in Fig. 218- $A_1$ . Subsequently three smaller ones arise caudad to the first three. Later, small bronchi arise in a more dorsal position; these are the so-called dorsobronchi.

During the formation of the bronchial branches, the mesobronchus has an S-shaped form. The posterior bend is the more pronounced. The terminal end is inflated and projects into a mass of mesenchymal tissue to form the primordium of the abdominal air sac. The central part is the expanded embryonic vestibulum. After the early stages, the development of the bronchial tree consists chiefly of the ramification and growth of its branches.

**The entobronchi.** After their appearance on the sixth day, the entobronchi grow by continual elongation. They are among the larger and more conspicuous divisions of the bronchial tree. In general, they are ventral in distribution; and the parabronchi that spring from their branches bend



around the mesial border of the lung and curve on to the dorsal surface to meet parabronchi coming from the ectobronchi.

The first entobronchus has an enlarged bladder-like extremity that lies above the bronchus (cf. Fig. 217- $E_2$ ). The distal end of the bladder soon proceeds to bifurcate (Fig. 217- $F_1$ ; Fig. 218- $A_1$ ). The anterior of the two buds is the beginning of the cervical air sac. The other is a lateral branch that will contribute the lateral moiety of the interclavicular air sac.

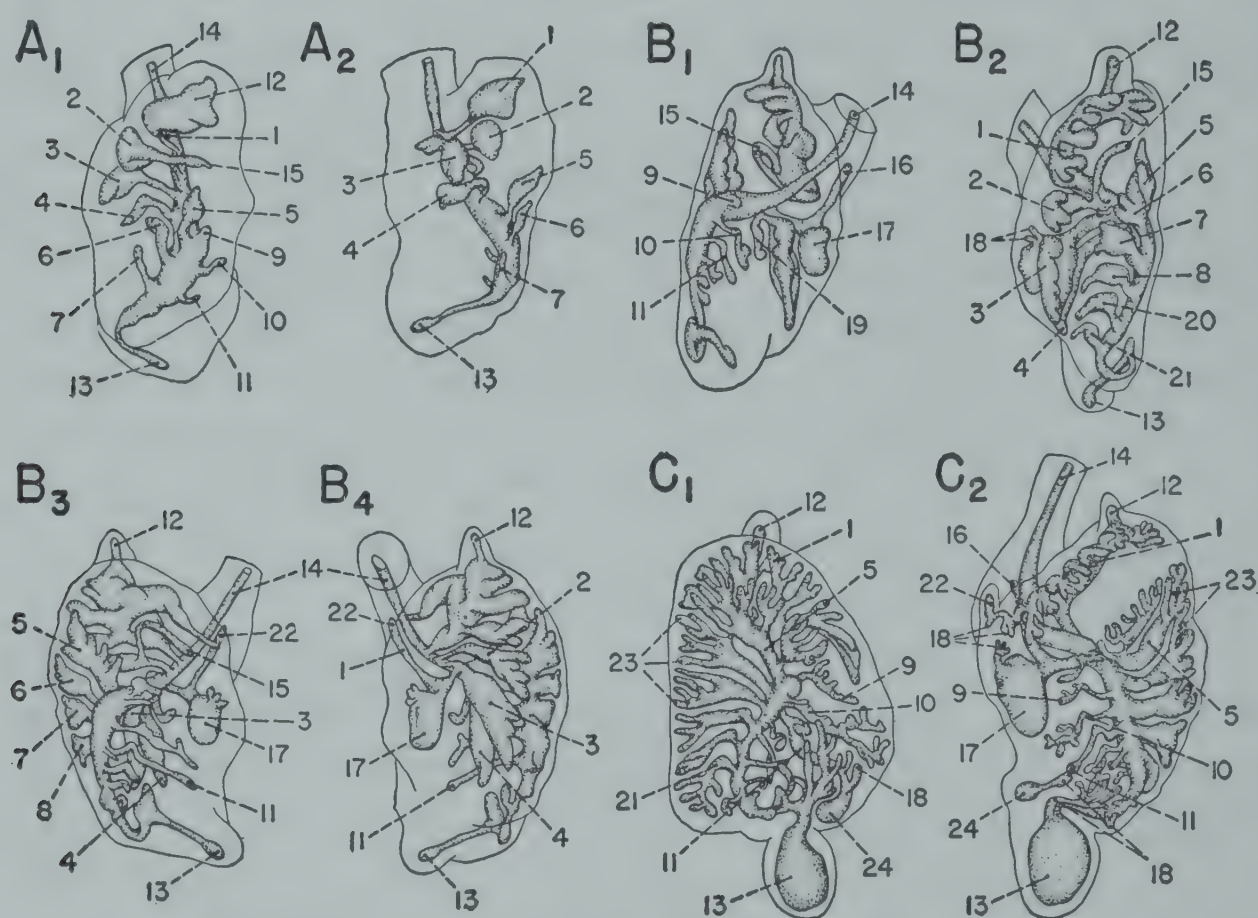


Fig. 218. Development of the bronchial system of the chick embryo's lung on the seventh to ninth days of incubation. (Redrawn with modifications after Locy and Larsell, 1916a.)

$A_1$ , dorsal view and  $A_2$ , mesial view of lung of approximately a 7-day embryo;  $B_1$ , ventral view and  $B_2$ , dorsal view of air injected right lung of an embryo early on the ninth day of incubation;  $B_3$ , lateral view and  $B_4$ , mesial view of the same embryo;  $C_1$ , latero-posterior view of right lung and  $C_2$ , lateral view of left lung of an embryo late in the ninth day of incubation. All  $\times 20$ .

1 to 4, entobronchi 1 to 4; 5 to 8, ectobronchi 1 to 4; 9 to 11, laterobronchi 1 to 3; 12, cervical air sac; 13, abdominal air sac; 14, bronchus; 15, dorsal branch of second entobronchus; 16, mesial moiety of interclavicular sac; 17, anterior intermediate air sac; 18, recurrent bronchi; 19, transverse branch of fourth entobronchus; 20, fifth ectobronchus; 21, sixth ectobronchus; 22, lateral moiety of interclavicular air sac; 23, parabronchi; 24, posterior intermediate air sac.

The second entobronchus is similar in origin to the first but is smaller. It gives off a slender dorsal ramus on the sixth incubation day. On the ninth day, the other extremity of this entobronchus has three lobes (Fig. 218- $B_2$ ) that become parabronchi and eventually supply the mesial region of the adult lung.

The third entobronchus arises more mesially than the first two (Fig.



217- $E_1$ ). It extends dorsally, then bends caudally. It has an expanded distal end (Fig. 218- $A_1$  and  $A_2$ ) from which two branches arise. The anterior branch forms the mesial moiety of the interclavicular air sac, and the ventral branch becomes the anterior intermediate air sac. The stem of the interclavicular air sac further expands to form the syringeal air sacs. The main portion of the entobronchus elongates and becomes trilobed, and these lobes later give rise to the parabronchi of the middle portion of lung.

The fourth entobronchus is not connected with any air sacs. It arises from the mesobronchus at the point where the embryonic vestibulum begins. On the seventh day, this entobronchus is short and has an expanded end (cf. Fig. 217- $F_1$ ). On the following day, a sickle-shaped branch arises at its base (Fig. 218- $B_1$  and  $B_4$ ), and this becomes the conspicuous great transverse branch of the adult lung (Locy and Larsell, 1916a).

**The ectobronchi.** These branches of the bronchial tree arise from the middle portion of the mesobronchus at the region of the expanded embryonic vestibulum. They arise later than the entobronchi and are more mesial in position on the mesobronchus. None of the ectobronchi have direct connection with air sacs.

The first ectobronchus (cf. Fig. 217- $F_1$ ) is the anterior one and projects forward and upward. It arises on the seventh day, elongates rapidly, and gives off a ventral branch. Both growing tips continue to produce lobelike branches until, at the beginning of the ninth day, there are five (Fig. 218- $B_3$ ). These are the primordia of the parabronchi of the anterolateral and dorsal regions of the lung. The number of lobes increases.

The second ectobronchus also arises on the seventh day as a bud from the dorsomedial surface of the embryonic vestibulum. It begins to branch on the ninth day when its distal end divides into three lobes (Fig. 218- $B_1$  and  $B_2$ ). These are the primordia of the parabronchi, which, in the adult, supply the dorsomedial face of the lung between the third and fourth ribs.

The four other ectobronchi are very similar in development, each being more medial than the preceding one. They all curve toward the caudal border of the lung. Their parabronchi supply the mesial region of the posterior half of the adult lung. A seventh ectobronchus may be present in the chick (Locy and Larsell, 1916a).

**The laterobronchi.** The six laterobronchi arise from the lateral wall of the embryonic vestibulum. The second and third appear first, at the stage of 6 days, and the first one forms a little later. They elongate ventrally and are prominent on the seventh day. By the ninth day, the second and third are bent caudally, and the second has divided many times to form the primordial parabronchi of the middle part of the ventrolateral lung region (Fig. 218- $C_1$  and  $C_2$ ). The third laterobronchus projects beyond the lung wall and gives rise to the posterior intermediate air sac, which appears on



the ninth day. The third laterobronchus also gives off a number of branches. The first laterobronchus, which is the most anterior one, is a bud on the seventh day. It grows ventrally and gives off branches that produce short parabronchi. In the adult, these anastomose with the deeper branches of the first entobronchus and with parabronchi of the second entobronchus. The fourth and fifth laterobronchi arise from the posterior part of the mesobronchus caudad to the vestibulum. They appear on the eighth day and eventually form parabronchi extending to the posterior lateral region of the lung.

*The dorsobronchi.* The dorsobronchi are the smallest and most numerous of the bronchi arising from the mesobronchus. Some even arise on the stems of the latero- and ectobronchi; others are so small that they seem to belong to the tertiary bronchi, although, according to their origin, they must be classed as secondary bronchi.

There are two groups of dorsobronchi, four or five larger ones and nearly twenty smaller ones. The larger dorsobronchi arise on the eighth day of incubation as spurlike projections from the lateral side of the mesobronchus, just dorsal to the bases of the first, second, and third laterobronchi (Fig. 218-B<sub>3</sub>). Subsequently, many more buds appear on the mesobronchus, and dorsobronchi are so numerous at 11 days, that they appear to obscure other air tubes of the lung.

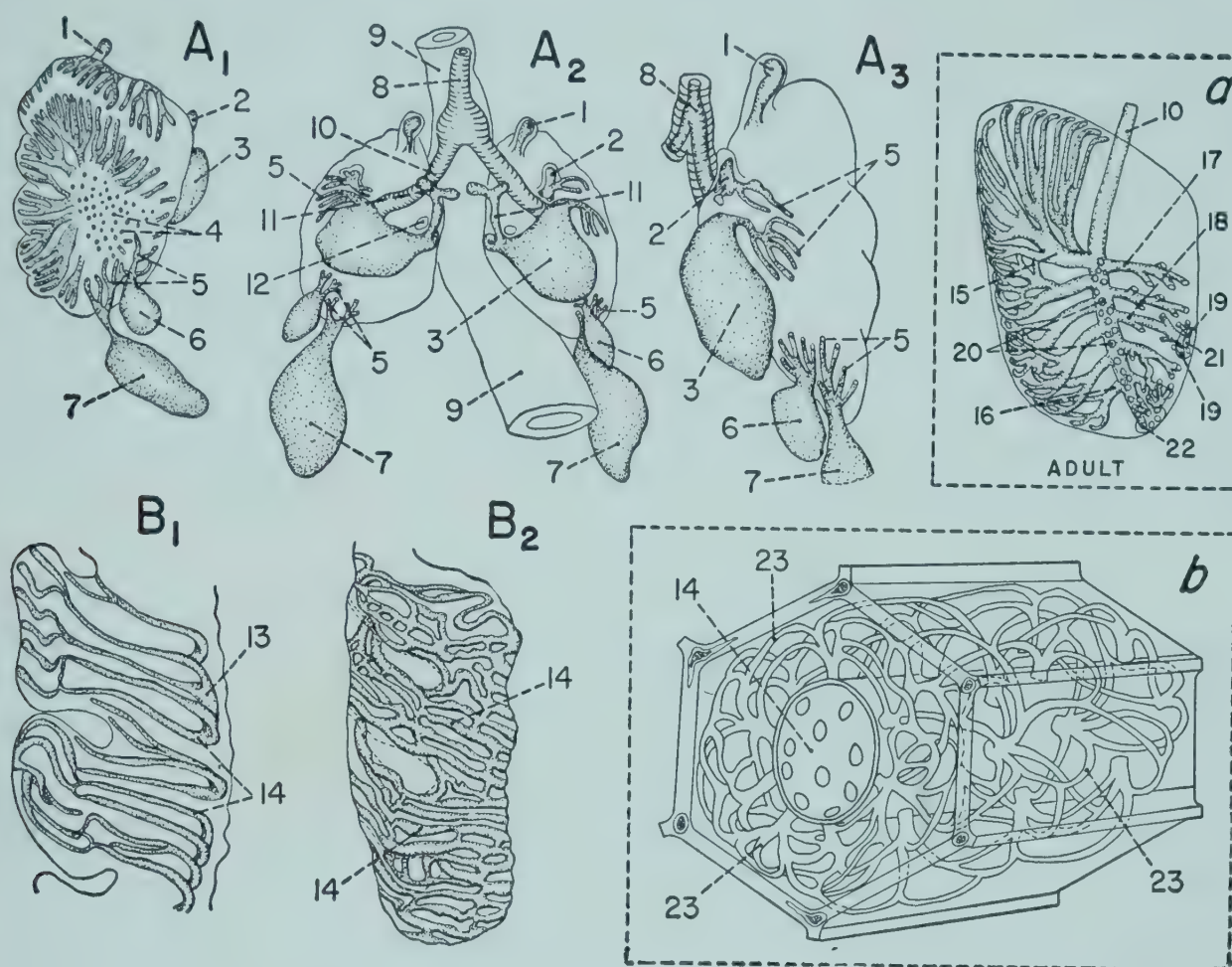
The dorsobronchi are arranged in three rows on the mesobronchus, with the largest dorsobronchi (which develop first) in the middle row and the smaller ones in the two outer rows (Fig. 219-Insert *a*). The branches of all the dorsobronchi project toward the surface of the lung, where their parabronchi form a clearly defined circular network in the center of the dorsal face of the lung. In a surface view of the 10.5-day chick lung (Fig. 219-A<sub>1</sub>), the dorsobronchi appear in end view near the center of the lung and in side view over the rest of the lung.

*The parabronchi.* This last division of the tube system of the lungs is the tertiary order, whereas the secondary branches comprise all those tubes that open directly on the primary, or mesobronchus. The outer tips of the fanlike system of larger tubes are the parabronchi, which are cylindrical tubes of small, nearly uniform caliber. They form an anastomosing network characteristic of the avian lung. Together with the air capillaries that spring from them, they comprise a large part of the mass of the lungs.

Development from the tenth day on consists principally of the establishment of the anastomosing network and the resultant bronchial circuit. The fine tips of the ecto- and entobronchi approach one another until, by the eleventh day, only a small strip of mesenchyme separates the two groups. On the twelfth day the parabronchial tips are almost in direct contact. They bifurcate, forming slender twigs, which then unite with twigs coming from the opposite direction. Complete union is achieved by the fifteenth



day of incubation, but the connecting parabronchi are very slender (Fig. 219-B<sub>1</sub>). The line of the parabronchial junction is seen as a distinct strip along the dorsal margin of the adult lung. After the eighteenth day of development further connections are established between parabronchi in other parts of the lung, both externally and internally (Fig. 219-B<sub>2</sub>).



**Fig. 219.** Development of the bronchial tree of the chick embryo's lung between the tenth and eighteenth days of incubation. (Redrawn with modifications A<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, Insert b, after Locy and Larsell, 1916a; A<sub>2</sub>, A<sub>3</sub>, Insert a, after Locy and Larsell, 1916b.)

A<sub>1</sub>, dorsal view of right lung of a 10.5-day embryo ( $\times 13$ ); A<sub>2</sub>, ventral view and A<sub>3</sub>, lateral view of pulmonary system of a 10.5-day embryo ( $\times 13$ ); B<sub>1</sub>, anastomosis of parabronchi in part of mesial facet of lung of a 15-day embryo ( $\times 20$ ); B<sub>2</sub>, similar view of an 18-day embryo ( $\times 12$ ).

Insert a, adult lung, dorsolateral view with severed bronchial stems ( $\times 6$ ); Insert b, diagram of anastomosis of air capillaries ( $\times 40$ ).

1, cervical air sac; 2, lateral moiety; 3, anterior thoracic air sac; 4, dorsobronchi; 5, recurrent bronchi; 6, posterior thoracic air sac; 7, abdominal air sac; 8, trachea; 9, esophagus; 10, bronchus; 11, mesial moiety; 12, pulmonary vein; 13, entobronchus 2; 14, parabronchus; 15, ectobronchus 1; 16, ectobronchus 6; 17, laterobronchus 1; 18, laterobronchus 3; 19, recurrent orifices; 20, dorsobronchi; 21, orifice of posterior thoracic sac; 22, orifice of abdominal air sac; 23, capillary.

**The air capillaries.** The ultimate branches in the increasingly fine system of air tubes are the air capillaries. After the ninth day, the parenchyma of the lung is arranged in columns around parabronchi. The columns are hexagonal in cross section (Fig. 219-Insert b), and the circular parabronchi are concentrated in the cores of the columns. Minute branches project from the parabronchi into the lung parenchyma; these branches are the



air capillaries which appear between the fourteenth and sixteenth day of development. At first they end as blind tubes, but they anastomose profusely before the twenty-first day (*Locy and Larsell, 1916b*).

The air capillaries are formed as a result of the expansion of the tips of the parabronchial tubes. At 16 days in the chick, the tips branch, widen, form vesicles by acquiring a lumen, and thus represent alveoli (*Juillet, 1911*). As the lumina widen, the epithelium lining the vesicles becomes more compressed. The protoplasm is pushed back until the nucleus is left bulging into the lumen. At the eighteenth day, just before the beginning of respiration, cytoplasmic granules are concentrated at the poles of the nuclei. By the seventeenth day, droplets appear in the lumen but they disappear before the onset of true respiration. The lumina of the parabronchi expand when air enters (*Harada, 1941*). Between the nineteenth and twentieth day the air capillaries anastomose, forming an intricate network and completely enveloping the parabronchi (*Juillet, 1911*).

### *Contractile Movements*

In the tissue cultures of embryonic bronchial tubes, Lewis (1924) noted spontaneous rhythmical contraction resembling that of the muscles of the chick amnion and intestine. Observations made on cultures of bronchial tubes of embryos incubated 9 to 11 days showed that muscles contract the lumen and to some extent shorten the tube, resulting in a wavy outline. The contraction sometimes closes the tube throughout its length, and in other cases is too weak to produce much effect. Often, feeble contractions alternate with regular, more violent contractions which close the tube about once a minute. Contraction is effected by the scattered bands of smooth muscle which are arranged in a single layer just outside the epithelial lining. There are also a few bundles of smooth muscle passing along the length of the tube.

In the lungs of embryos incubated more than 11 days, the bronchial tubes are no longer straight, and the walls are pitted over their inner surfaces with hollow buds of epithelium. These diverticula also contain smooth muscle; and in cultures of lungs explanted after 12 to 16 days' incubation and kept at 40° C. the smooth muscle exhibits constant movement.

The contraction of the muscles moves the blood back and forth in the blood spaces along the bronchial tubes. Occasional loose cells and debris in the lumen of the tube are driven back and forth with each contraction and relaxation.

### *Movement of Air*

The manner of ventilation in the definitive lung was studied by Hazelhoff (1951) and it was definitely shown that regulation of the passage of air is not by the alternate opening and closing of a large number of valves



but is governed by principles of aerodynamics. It was shown that air current always flows in the same direction from dorsobronchi to parabronchi to ventrobronchi, which together make up a large part of the bird lung. During inspiration as well as expiration, the air flows in the direction named. The posterior air sacs produce the current and the anterior sacs are of slight importance in this respect. The renewal of air in the capillaries takes place entirely by diffusion.

The work of Brandes (1924) showed that the chambers which stored gas were entirely separate from those tubes which were involved as a respiratory surface. The storage organs were of two types, one for expired air, the other for inspired air. The posterior air sacs are for inspired air and from here the air goes to the capillaries of the lung, whose walls are the exchange surface. Then the air to be expired goes to the anterior sacs which open to the outside.

### The Air Sacs

In the adult avian lung there are five large, axial air sacs; the cervical, the interclavicular, the anterior thoracic, the posterior thoracic, and the abdominal air sac. All are paired except the interclavicular. The cervical and interclavicular arise anteriorly, while the other three pairs are on the ventral and caudal surfaces of the lungs (cf. Fig. 218-C<sub>2</sub>). In addition to these, there are the small syringeal air sacs.

**Embryogeny of the Air Sacs.** The first air sacs appear in the 6.25-day chick embryo and can be seen at this stage as projections of the lung wall. The abdominal air sac is seen in Fig. 217-F<sub>2</sub> which also shows the cervical sac as a bud from the first entobronchus. The anterior thoracic sac, with the mesial moiety of the interclavicular appended to it, appears as a bud of the third entobronchus. The interclavicular sac arises from two moieties on each side, the lateral and mesial, which come from different sources (*Locy and Larsell, 1916b*).

The air sacs as well as the bronchial tree are greatly enlarged by the ninth day. All five air sac primordia now project beyond the lung surface. The lateral moiety of the interclavicular sac has begun to form as the transverse branch of the first entobronchus. The mesial moiety is an anterior branch of the third entobronchus. An even larger, more caudal branch from the third entobronchus is the growing anterior thoracic sac. It is the largest of the embryonic air sacs. From its ventral anterior part, three papilla-like buds are the primordia of the recurrent bronchi of the anterior thoracic air sac; these buds are derived from a single bud formed during the latter part of the seventh day of incubation. Also on the ninth day, the posterior thoracic air sac appears as a projection from the third laterobronchus and is without recurrent bronchi (*Larsell, 1914*).

Between the ninth and tenth days, the recurrent bronchi grow steadily.



Several sacs have expanded greatly; the abdominal sac, for example, lies almost entirely outside of the lung (cf. Fig. 218-C<sub>1</sub> and C<sub>2</sub>). It has two groups of recurrent bronchi, a ventral group, twice bifurcated, and a dorsal group with an anterior and a lateral limb (cf. Fig. 218-C<sub>1</sub>).

By the eleventh day the air sacs are still larger, and the two moieties of the interclavicular sac are present (Fig. 219-A<sub>2</sub>). Figure 219-A<sub>2</sub> shows the enlarged recurrent bronchi of the anterior thoracic sac, and also the connection between the mesial moiety of the interclavicular sac and the anterior thoracic sac. Both of these sacs arise from a common canal that opens from the third entobronchus. The recurrent bronchi from the lateral moiety and the anterior thoracic air sac are shown in Fig. 219-A<sub>3</sub>.

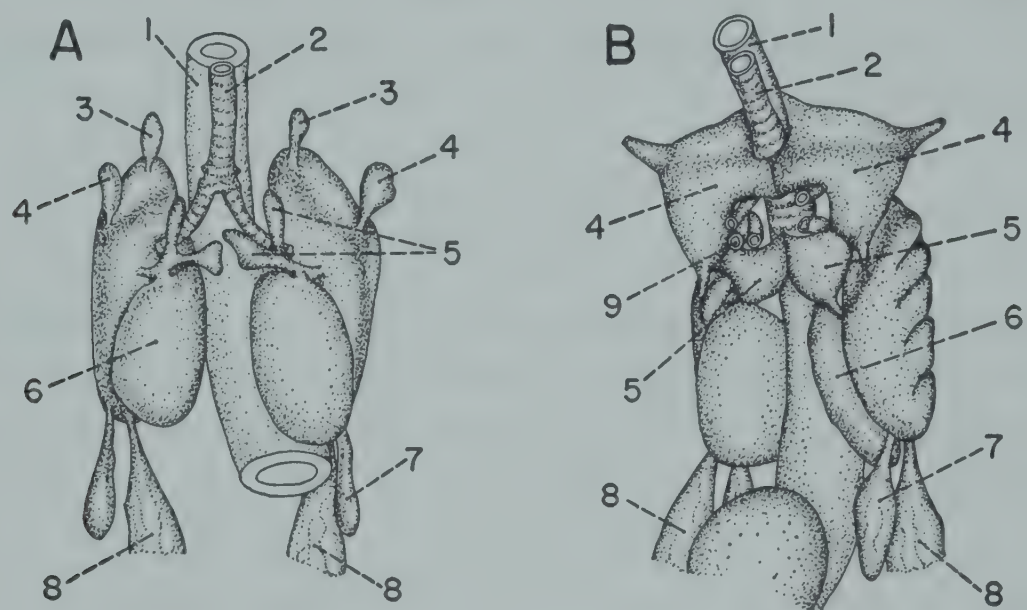


Fig. 220. Air sacs of the chick embryo. (Redrawn with modifications after Locy and Larsell, 1916b).

A, ventral view of air sacs and lungs of a 12-day embryo; B, lateroventral view of lungs and air sacs of a 15-day embryo. Both  $\times 15$ .

1, esophagus; 2, trachea; 3, cervical air sac; 4, lateral moiety of interclavicular sac; 5, mesial moiety of interclavicular air sac; 6, anterior thoracic sac; 7, posterior thoracic air sac; 8, abdominal air sac; 9, aorta.

From the twelfth to the fifteenth days the changes in most of the air sacs is simply enlargement by growth. During this time, however, the interclavicular sac reaches its azygous (unpaired) condition. At the end of the twelfth day the mesial moieties of this sac begin to approach each other in the interbronchial region as shown in Fig. 220-A. The common opening of the mesial moieties and the anterior intermediate sac into the lung is also shown. Very quickly the mesial moieties fuse, and they then unite with the greatly expanded lateral moieties of the interclavicular sac (Fig. 220-B). The wall between the fused mesial moieties disappears on the first day after hatching. The septum between the mesial and lateral moieties disappears on the eighteenth day (Locy and Larsell, 1916b).

There are two types of air sacs, based on the number of their orifices. Where a single bronchus opens into an air sac, the sac is said to be mono-



bronchial. Polybronchial sacs usually have several openings, often including recurrent bronchi.

**The Recurrent Bronchi and the Air Sacs.** The recurrent bronchi are outgrowths from the air sacs rather than extensions of the bronchial tree of the lung. In the course of development they lead into the lung parenchyma and unite with twigs of the bronchial tree. In so doing they establish a complete circuit with the air passage within the lung. They bear the same relation to the air sacs that the parabronchi bear to the respective secondary branches from which they have their origin. By means of the recurrent bronchi and their anastomoses with other branches of the bronchial circuits, the air sacs are brought into communication with all parts of the lung. The air sacs are thus directly connected to the bronchus and mesobronchus and also have recurrent communications through the recurrent bronchi.

By the sixteenth day the recurrent bronchi are well formed (cf. Fig. 219-A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>). The distal tips of the longest recurrent bronchi of the abdominal air sac have anastomosed with the lateroventral parabronchi of the first entobronchus. The recurrent bronchi of the two other air sacs anastomose during the sixteenth day with parabronchi in parts of the lung adjacent to them. By the eighteenth day the recurrent bronchi have attained the relationships to other parts of the bronchial tree which they exhibit in the adult lung.

The change to the adult condition is brought about by the expansion of the sacs. The abdominal sacs come to fill the abdominal cavity and partly surround the viscera. Around the fourteenth incubation day these sacs fuse with the peritoneum. The posterior thoracic sacs do likewise. The anterior thoracic sacs expand and fuse with the lining of the thoracic cavity. The cervical sacs grow rapidly after the twelfth day of embryonic growth and fuse with the pleura of the neck by the nineteenth day (*Locy and Larsell, 1916b*).

**Muscle Activity.** In culturing air sacs taken from chick embryos of various stages of incubation, Lewis (1924) found that the smooth muscle underwent continued movement for many days in culture. The air sacs are lined with endoderm and covered by a loose reticulum of smooth muscle fibers. Each muscle bundle exhibited contraction and relaxation, but seldom at the same time or the same rate as its neighbors, so that the entire surface of the piece was continually writhing without coordinated contraction of the whole piece.

## RESPIRATORY MOVEMENTS

Movements of the embryo within the intact shell membrane have long been observed. Early in the incubation period, this activity is purely a muscular reflex and may include such actions as body vibration, head



lifting, motion of trunk, beak, toes, and eyeballs (*Preyer, 1885*). Toward the end of incubation such features as the increase of carbon dioxide tension and drying of the surrounding fluids stimulate active pulmonary respiration.

**Initiation of True Respiratory Movements.** Respiratory movements seem to be initiated at the time that the allantois ceases to serve adequately as a respiratory mechanism. The trigger mechanism that starts respiratory movements may be a condition of anoxemia and carbon dioxide excess (*Windle, Scharpenberg, and Steele, 1938*). Direct observation of the color change in the blood of the extraembryonic circulation tends to confirm the theory that elevated carbon dioxide tension is an essential factor in initiating pulmonary respiration (*Kuo and Shen, 1937*). On and after the fifteenth day of incubation the blood vessels are easily observed through the inner shell membrane and the contained blood becomes darker. Kuo and Shen (1937) feel that drying of the skin may also serve as a factor in promoting respiratory movements. True respiratory movements do not occur until the amniotic fluid has dried; furthermore, injection of warm isotonic solution into the dry amniotic cavity reduces the rate of respiration.

According to Romijn (1948), lung ventilation begins at the moment the air chamber is perforated by the beak of the embryo. Romijn was not able to produce pronounced changes in the rhythm of movement by altering the carbon dioxide and oxygen pressure, and he concluded, therefore, that chemical regulation of respiration at this stage is poorly developed.

**Analysis of Movement.** Respiratory movement begins on the sixteenth day of chick incubation at the earliest (*Kuo, 1932b*). The majority of embryos exhibit initial respiratory movement on the eighteenth day, as shown in the accompanying table.

Age of Embryo (days)	Embryos Showing Initial Respiratory Movement (per cent)
16	3.3
17	16.2
18	55.7
19	24.8

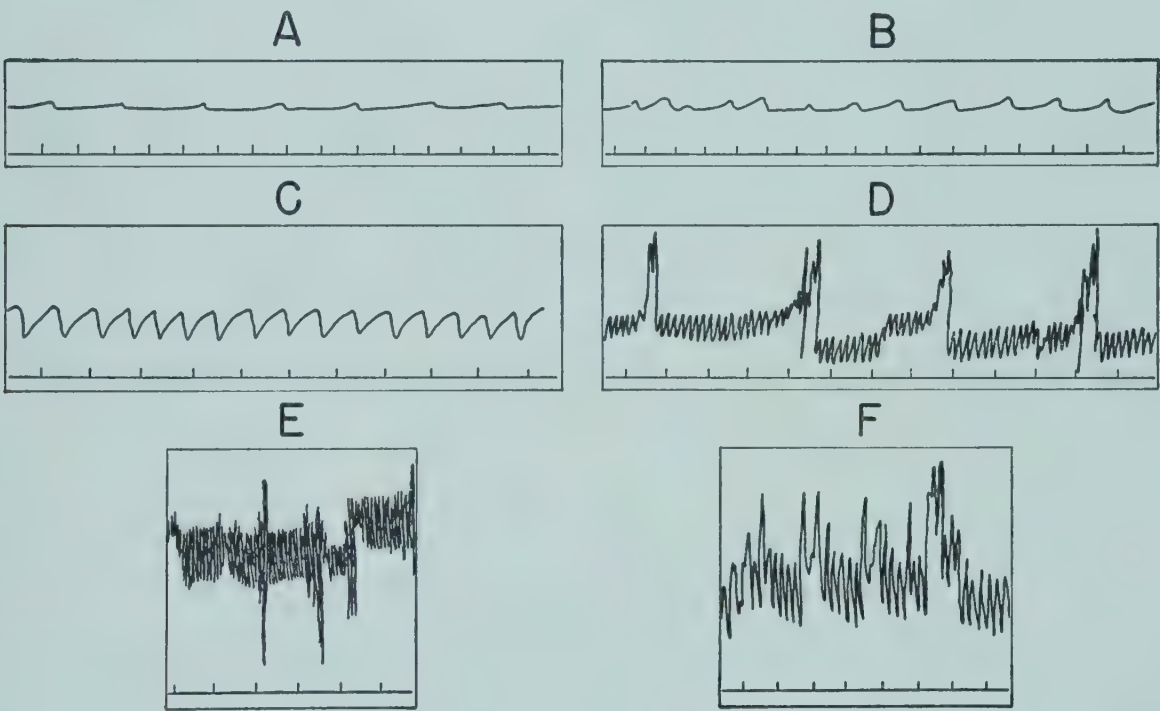
As the period of incubation progresses, the frequency of respiratory movements as well as the amplitude increases, as shown in the accompanying table (*Kuo and Shen, 1937*).

The same data are shown graphically in Figure 221-A to F. The kymograph tracings shown illustrate the increasing frequency and amplitude of respiratory movements from the seventeenth day on. The downstrokes represent the quick inspiration followed by more gradual expiration. In



Age of Embryo (days)	Respiratory Movement	
	Frequency (number per minute)	Amplitude (mm.)
17	5	3
18	17	5
19	46	7
20	84	8
21	91	10
Hatched	82	15

the experiment depicted, embryos were removed from the shell and fastened to a board in order to obtain the readings (*Kuo and Shen, 1937*). The abnormal condition thus imposed on the embryo might produce some deviation from the normal intraegg behavior.



*Fig. 221. Kymograph records of the normal respiratory movements of the chick near the time of hatching. (Redrawn with modifications after Kuo and Shen, 1937.)*  
A, 17 days; B, 18 days; C, 19 days; D, 20 days; E, 21 days; F, after hatching.  
The downstrokes in the kymograph tracings represent inspiratory movement.

Respiratory movements are at first irregular in rate and uneven in amplitude, as shown by kymograph records made by inserting a glass tube and tambour into a hole in the shell. Soon after 18 days air is presumably being breathed. Shortly before the time of hatching the rate of breathing becomes regular and increases as shown in the accompanying table (*Windle, Scharpenberg, and Steele, 1938*).

Age of Embryo (days)	Respiratory Movements (no. per min.)
18	80-90
21	100-120



Experiments on the influence of carbon dioxide and oxygen tension on the respiratory rate of the 20-day chick showed, rather inconclusively, that carbon dioxide in excess of oxygen suppresses the respiratory rate. Excess oxygen alone does not seem to alter respiratory rate. But when the amount of oxygen available to the newly hatched chick is decreased, a sharp drop in the respiratory rate is noted (Fig. 222) (Windle, Scharpenberg, and Steele, 1938). It has been shown that at the end of the nineteenth day carbon dioxide content is about 5 per cent and the oxygen about 15 per cent of the gas content of the air chamber (Romijn, 1948).

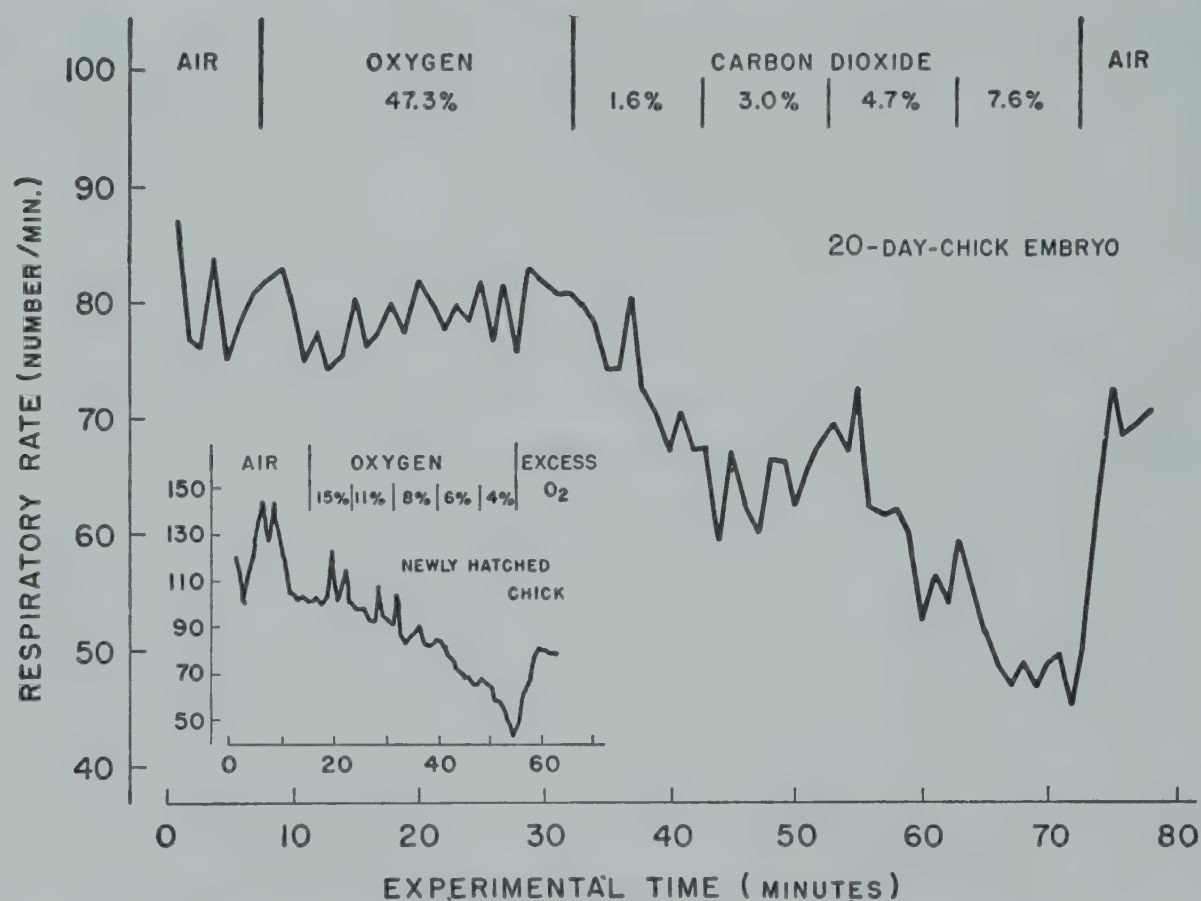


Fig. 222. The effect of carbon dioxide and oxygen tension on respiratory rate of the chick embryo. (Redrawn with modifications after Windle, Scharpenberg, and Steele, 1938.)

An analysis of the rate of oxygen consumption throughout the incubation period reflects, to a certain degree, the pattern of respiratory movement. Between the seventeenth and twentieth days, when respiratory movements become more regular, oxygen consumption decreases. At hatching time, the rate of oxygen consumption increases markedly (Romanoff, 1941). Of course, it is reasonable to assume that oxygen consumption is based on the degree of physical activity, and that both are at a high level at hatching.

**Phases of Respiratory Movement.** Closer analysis of the respiratory movements shows three rather distinct periods of activity. The first is in the thoracic region and is a muscular type of movement which may be accompanied by movement of the trunk. These motions occur prior to the seventeenth day and are not true respiratory movements (Kuo and Shen, 1937; Preyer, 1885).



The second stage of movement occurs between the seventeenth and eighteenth days and involves principally the thorax. The movements that appear at this time are considered as true respiratory motions, although little or no air is inhaled or expelled, for the embryo is still surrounded by a liquid medium (*Kuo and Shen, 1937*).

If the chick is removed from the egg on the seventeenth day, it makes frequent energetic respiratory movements of opening and closing the bill and expanding the thorax. On the eighteenth day, the exposed chick opens the bill wide and gasps for air (*Preyer, 1885*).

During the last 2 or 3 days of the incubation period, true pulmonary respiration takes place. At this time, the amniotic fluid surrounding the beak of the embryo has disappeared. With the onset of the third stage, an additional supply of oxygen is needed for hatching. Since the extraembryonic blood vessels are degenerating and the little air in the amniotic cavity is used, the embryo must pierce the inner shell membrane in order to utilize the air in the air chamber (*Kuo and Shen, 1937*). In many cases, true air-breathing has been observed before the embryo has perforated the outer shell membrane, which alternately bulges and sinks inward as the chick breathes.

The rate of respiratory movements on the nineteenth day ranges from 72 to 90 inspirations per minute (*Preyer, 1885*). These are rhythmic movements. Strong inspirations may be forced by pinching the foot. On the twentieth day respiratory movements generally exceed 90 per minute. Such movement is recognizable by rhythmic swinging of the air chamber.





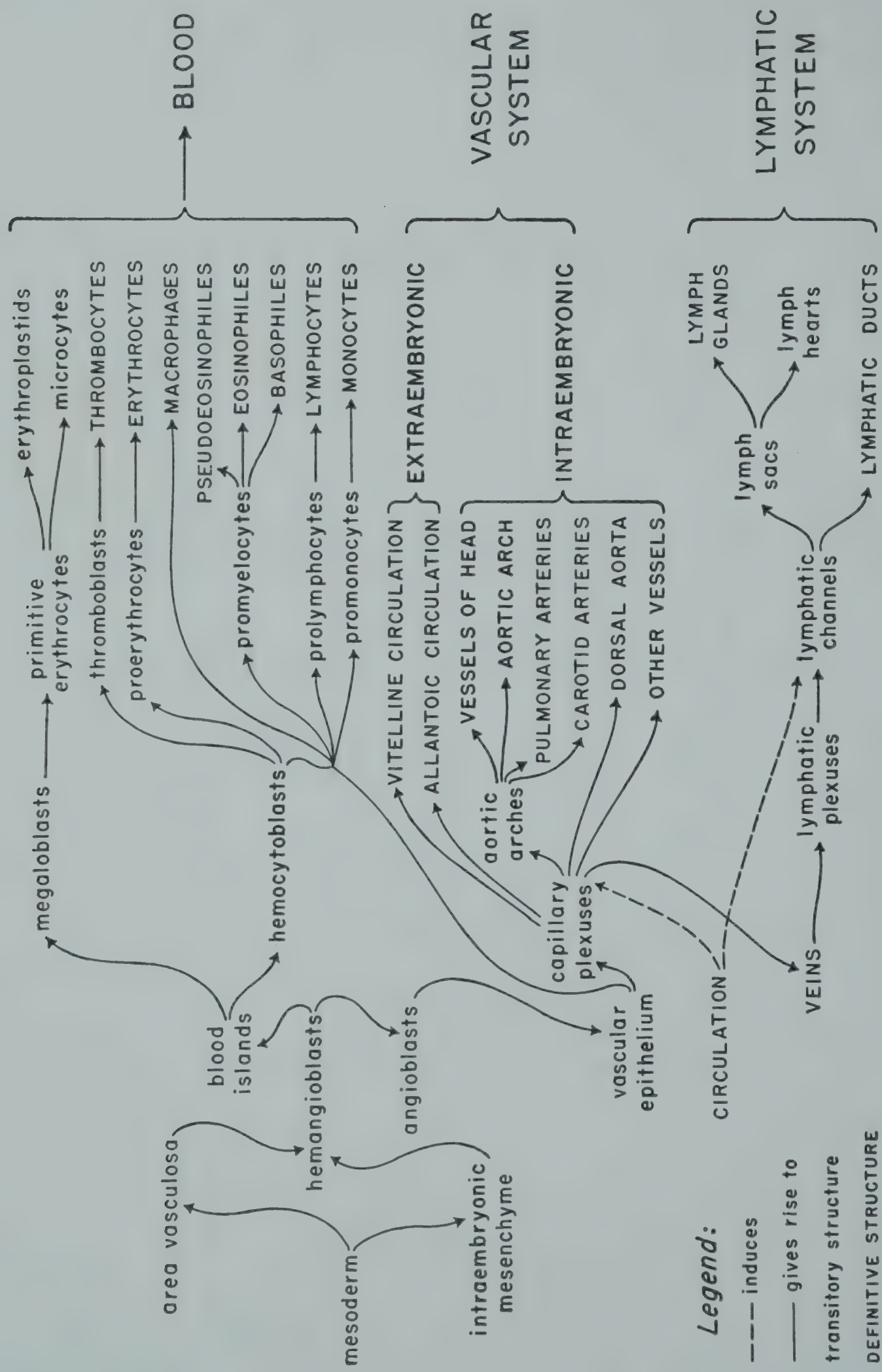


## CHAPTER EIGHT

# The Hematopoietic, Vascular, and Lymphatic Systems

*The blood requires circulation;  
It flows through arteries and veins;  
Its parts thus reach their destination . . .  
And lymph, as guard, here too remains.*





DEVELOPMENT OF THE HEMATOPOIETIC, VASCULAR, AND LYMPHATIC SYSTEMS IN THE AVIAN EMBRYO



# THE HEMATOPOIETIC, VASCULAR, AND LYMPHATIC SYSTEMS

Except during the first few days of development, the tissues of the avian embryo receive nutriment in the same manner as those of the adult, that is, via the blood stream. The yolk is the source of food, and it is not surprising, therefore, that blood and blood vessels are formed in the yolk sac before they appear in the embryo's body. By the time the embryo is equipped with a primitive vascular system and a beating heart, there is a well-developed network of yolk sac vessels (see Chapter 13). These vessels contain blood cells, blood plasma, and material absorbed from the yolk, and they are continuous with the intraembryonic vessels. Soon after the heart starts to pulsate, the contents of the extraembryonic vessels enter the intraembryonic vessels.

In the beginning, hematic and vascular tissues differentiate simultaneously in the yolk sac from the same primordial mesoderm. Subsequently, blood cells and endothelial cells both increase through cell division. The yolk sac is the chief source of blood cells for the first half of the incubation period and continues to produce them almost until the hatching date.

Within the embryo, endothelial tissue at first differentiates *in situ* much as it does in the yolk sac, but with the difference that little or no hematopoiesis accompanies its development. In the embryo's body, hematopoiesis is largely confined to organs that do not make their appearance until the second half of incubation; these organs gradually surpass the yolk sac in the production of blood cells.

The vascularization of the embryonic body begins with the establishment of a few primitive blood vessels, of which some are permanent and others only temporary. The process of local differentiation that produces these vessels appears to be of limited duration; by far the largest portion of the vascular system is formed through the growth and extension of pre-existing endothelial tissue. So, also, is the system of lymphatic vessels which returns fluid from the tissues to the blood stream, although the capacity to proliferate lymphatic endothelium is confined to the veins and is not shared by the arteries.

The formation of blood vessels proceeds with great rapidity. The tissues of the body are invaded by capillary plexuses at a relatively early period,



and from these plexuses, larger vessels arise. The size and the course of the blood channels are determined by many conditions. As conditions change, the blood vessels alter their position; or they are required to carry a larger or a smaller load, and consequently they enlarge or atrophy. Yet the definitive vascular pattern becomes apparent by the midpoint of incubation, in most parts of the body.

### EARLY BLOOD AND VESSEL FORMATION

Vascular endothelium and blood cells are both derived from the extra- and intraembryonic mesenchyme and ultimately, therefore, from the mesoblast (*Remak*, 1855; *Afanasieff*, 1866; *Klein*, 1871; *Balfour*, 1873c; *Rauber*, 1877; *Kölliker*, 1884; *Stricht*, 1892, 1893; *Drasch*, 1894; *Schauinsland*, 1906; *Danchakoff*, 1908a, 1908b; *Kiyono and Nakanoin*, 1919–20; *Sabin*, 1920) which grows out from the primitive streak between the epiblast and the hypoblast of the early blastoderm (see Chapter 3). Experimental evidence of mesoblastic origin is provided by the finding that blastoderms subjected to extreme chilling before incubation developed neither primitive streak, mesoblast, nor area vasculosa (*Grodzinski*, 1933). Furthermore, isolation of the primitive streak from the extraembryonic blastoderm prevented the formation of blood vessels external to the embryonic body (*Hahn*, 1909; *Miller and McWhorter*, 1914); and destruction of part of the primitive streak caused deformation of the area vasculosa at the same level (*Patterson*, 1909a).

It should be noted, however, that some embryologists have suggested origins other than mesoblastic. *Goette* (1874), *His* (1876b), and *Vialleton* (1892a) believed that a special portion of the blastoderm, the “parablast,” of white yolk origin, grew out from the germ wall into interstices in the mesoblast and gave rise to the area vasculosa and subsequently to the connective and vascular tissues and blood. *Disse* (1879) proposed that the blood and vessels were the product of mesoblastic cells which were in turn derived from endoderm located on the periphery of the blastoderm. The participation of endoderm in blood and vessel formation was also affirmed by *Rabaud* (1896).

In the opinion of *Murray* (1932), the common anlagen of the vascular and hematic tissue of the area vasculosa arise through the self-differentiation of certain mesenchymal cells which are either predetermined or “strongly biased” in this direction while still within the primitive streak. *Murray* (1932) found that essentially normal hematopoiesis can occur in explanted tissue from the posterior three quarters of the definitive primitive streak, from the area pellucida lateral to this portion of the streak, and from the area opaca as far anterior as the cephalic end of the streak. The observations of *Rudnick* (1938a, 1938c) tend to corroborate this distribution of blood-



forming potency. Shipley (1916) and Tagaki (1931) also demonstrated the blood-forming potencies of explants taken from the area opaca at a time prior to the appearance of the first "blood islands," the forerunners of vessels and blood.

These "blood islands" (of Wolff) may become visible in the area opaca before the head-process is full-grown (*Rückert and Mollier, 1906; Weber, 1907b*) or not until the 1-somite stage (*Tur, 1907*). After the appearance of the first somite, they impart a mottled aspect to a horseshoe-shaped region of the area opaca caudal and lateral to the area pellucida. This mottling is caused by localized thickening of the mesoderm. Sabin (1920) stated that the mesoderm in the thickened spots remains undifferentiated until the 2- to 4-somite stage. Clumps of tissue then become apparent in the deeper layers of mesoderm (Fig. 223-A), usually directly beneath the septae separating the vesicles that comprise the exocoelom at this time. These clumps of tissue give rise both to blood cells and to vascular endothelial cells and are most appropriately called hemangioblasts (*Murray, 1932*); it has been suggested that the term "blood islands" should be reserved for the constituents of the hemangioblasts which produce blood cells (*Sabin, 1917a*) and "angioblasts" for the forerunners of vascular endothelium (*Murray, 1932*).

Hemangioblasts are at first syncytial in nature (*Danchakoff, 1908b*), although nuclei and cell boundaries are demonstrable in appropriately fixed and stained preparations (*Sabin, 1920*). The cells differ from those of the primitive mesoblast in that they are more basophilic, more refractile, and more granular (*Sabin, 1920; Sugiyama, 1926*). Their cytoplasm has a greater affinity for stains and their nuclei are rounder (*Jolly, 1939-40*).

Hemangioblasts differentiate first about midway between the anterior and posterior limits of the area opaca, close to the anterior border of the mesodermal layer. By the 4-somite stage they have appeared in the posterior region, where they are always most massive (*Vialleton, 1892b; Sabin, 1920*). The formation of hemangioblasts in the area opaca may be considered as indicating the initial differentiation of the area vasculosa of the extra-embryonic region (see Chapter 13).

Similar clumps of cells begin to appear within the area pellucida almost as soon as in the area opaca; according to Jolly (1939-40), they are visible even before the first somite forms. Sabin (1920) remarked their presence at the 2- to 3-somite stage, but added that they are not abundantly present until the 5-somite stage. In the 6- to 8-somite embryo, they are most numerous in the region of the future vitelline artery, at a level lying between Hensen's node and the last pair of somites (*Jolly, 1939-40*).

At the 5-somite stage, the first angioblasts—representing the future endocardium—appear within the primitive embryonic body. Others along the



lateral margins of the somites are the forerunners of the dorsal aortae (Sabin, 1920).

The extraembryonic hemangioblasts unite into a plexus of cellular cords (Fig. 223- $B_2$  and  $F$ ) that become blood vessels containing blood cells and plasma (Stricht, 1892; Danchakoff, 1908b; Sabin, 1920). This transformation of the solid cellular aggregates begins in the outermost zone of the area vasculosa (Fig. 223- $B_1$ ) at some time between the 5-somite (Sabin, 1920)

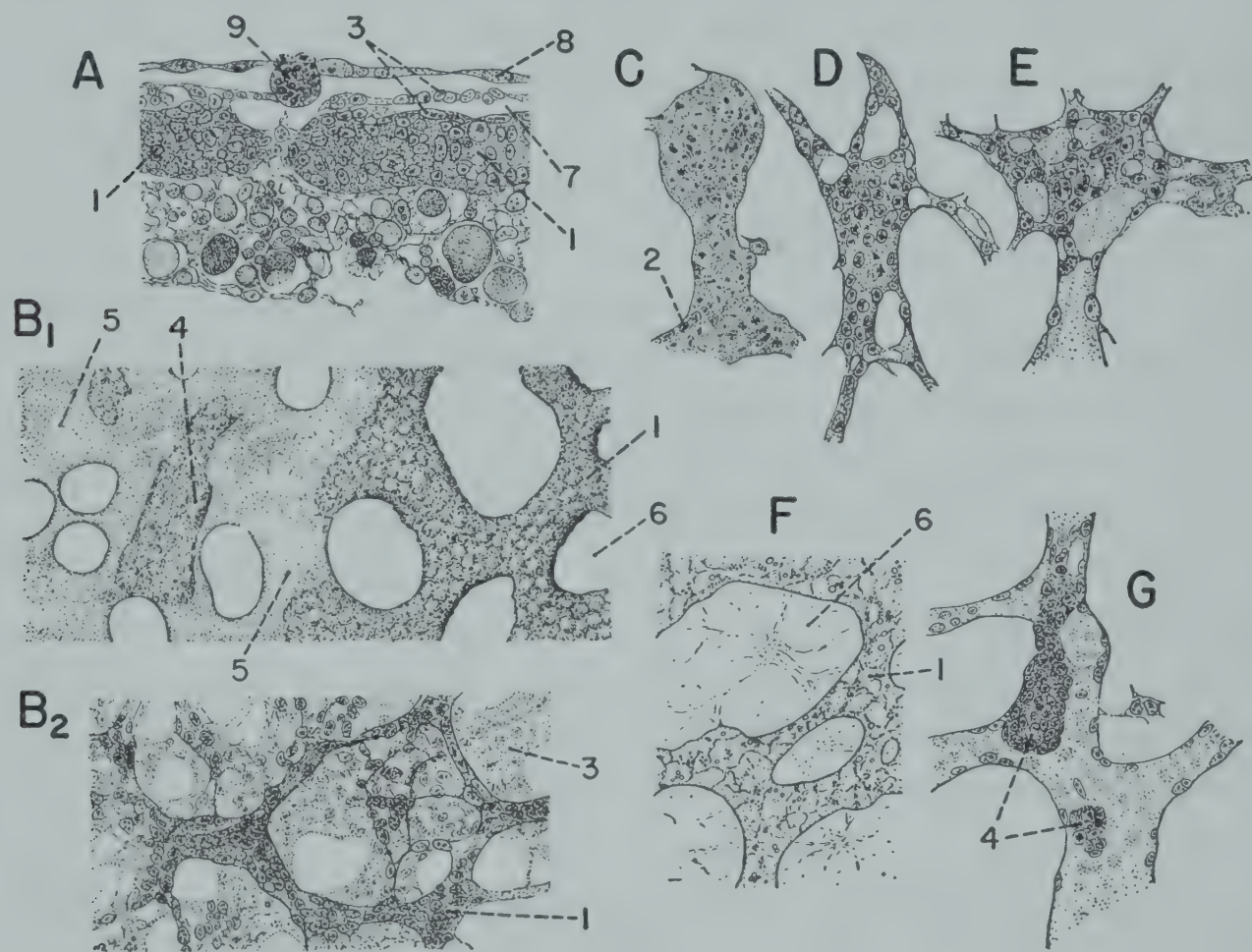


Fig. 223. Hemangioblasts in the yolk sac of the 2-day chick embryo. (Redrawn with modifications after Sabin, 1920.)

A, section through the posterior portion of the area opaca of a 4-somite chick embryo, showing the differentiation of the primitive mesoderm into the two layers of cells forming the coelom and the more ventral clumps of solid hemangioblasts ( $\times 250$ );  $B_1$ , the transition zone between the inner and outer zones of the area opaca of the 5-somite chick, showing the fully differentiated vessels of the outer zone (left side in the drawing) leading over into the solid hemangioblasts of the inner zone (right side in the drawing); blood islands can be seen attached to the vessel walls ( $\times 150$ );  $B_2$ , drawing of a plexus of hemangioblasts seen against the coelom in the area pellucida of the 5-somite chick embryo ( $\times 150$ ); C, D, and E, masses of hemangioblasts from the 11-, 13-, and 14-somite chick embryo, respectively ( $\times 250$ ). In C the hemangioblasts are entirely solid and show no sign of liquefaction; in D the center of the mass is solid, but vacuoles are forming around the edges, leaving an endothelial border; in E, taken from the area of aorta formation, liquefaction of the cytoplasm is extensive; F, bands of hemangioblasts in the area pellucida of the 13-somite chick as they appear in the living specimen ( $\times 150$ ); G, an angioblast from the aorta region of the 14-day chick embryo which has developed hemoglobin and is forming a blood island instead of liquefying ( $\times 250$ ).

1, hemangioblast; 2, endothelial cell; 3, mesoderm; 4, blood island; 5, lumen of vessel; 6, interspace; 7, coelom; 8, ectoderm; 9, wandering endodermal cell.



and the 7-somite stage (*Rückert and Mollier, 1906*) and proceeds centripetally. It becomes apparent in the anterior zone of the area pellucida by the 6- to 9-somite stage and in the posterior zone at about the 14-somite stage (*Sabin, 1920*).

Vascular endothelium is formed by the peripheral cells of the hemangioblastic masses (Fig. 223-C, D, and E; Fig. 224-A<sub>1</sub> and B). The liquefaction of the centrally located cells gives rise simultaneously to the vessel lumina and to blood plasma (cf. Fig. 223-C, D, and E). There has been some disagreement, however, as to whether cavitation occurs intercellularly (*Remak, 1852; Afanasieff, 1866; Kölliker, 1883; Thoma, 1893; Rückert and Mollier, 1906*); or intracellularly (*Klein, 1871; Balfour, 1873c; Sabin, 1920*), that is, by means of the cytoplasmic vacuolization; from observations of the duck (*Anas platyrhynchos*) blastoderm, Weber (1907a) concluded that it takes place in both ways. Endothelial cells, therefore, may represent flattened cells (*Coste and Delpech, 1833; Drasch, 1894*) or the remnants of partially cytolized cells (*Sabin, 1920*).

Many hemangioblast cells fail to liquefy and are enclosed within the newly formed blood vessels (Fig. 223-B<sub>1</sub> and G). Clumps of them may be seen (cf. Fig. 224-B) adhering to the endothelium (*Afanasieff, 1866; Stricht, 1892*). They are usually applied to the roof of a vascular space, since the ventralmost cells are the first to liquefy (*Rückert and Mollier, 1906; Jolly, 1939-40*). These clumps of cells have been called the true blood islands (*Sabin, 1920*), for they represent the portion of the hemangioblast that gives rise to blood cells. Blood islands are considerably less numerous in the area pellucida than in the area opaca, and very few of the newly formed capillary spaces of the area pellucida contain blood cells (*Remak, 1855*).

## BLOOD CELLS

Erythrocytes and leucocytes are both present in avian blood. The morphology of these elements reveals the phylogenetic relationship of birds to reptiles and to mammals.

In birds, as in reptiles, the red blood corpuscles are nucleated and have an elongated oval shape. The long diameter may be from one and one-half to two times as long as the short diameter (*Gulliver, 1875*). Within families, there is general direct relationship between the size of the erythrocytes (especially the long diameter) and the body size of the species (*Venzlaff, 1911*). The number of erythrocytes per cubic millimeter of blood also varies with the species (*Kalabukhov and Rodionov, 1934*), but in inverse relationship to body size (*Venzlaff, 1911*). Passerine birds, furthermore, have more red blood cells and a higher blood hemoglobin level than gallinaceous and other birds of comparable body weight (*Venz-*



laff, 1911; Sandreuter, 1951). In passerine birds weighing less than 500 gm., the red blood cell count tends to be as high as it is in human blood, or higher; whereas it approaches the human value in only the smallest gallinaceous birds (those weighing less than 100 or 200 gm.).

Avian white blood cells are of the same two general types as those found in mammalian blood, that is, nongranular leucocytes (lymphocytes and monocytes) and various granular leucocytes, or granulocytes (neutrophiles, eosinophiles, and basophiles). The lymphocytes are the predominant white cells (Grünberg, 1901; Cullen, 1903; Forkner, 1929), but their proportion of the total is very variable (Cullen, 1903). The most numerous of the granulocytes are cells which apparently correspond to the mammalian neutrophiles (heterophiles) but which contain eosinophilic rod-shaped or fusiform bodies of crystalloid appearance instead of amorphous neutrophilic granules. The avian granulocytes that are probably equivalent to mammalian eosinophiles resemble the latter, although their granules are less strongly acidophilic (Forkner, 1929); these cells may be termed pseudoeosinophiles. They are said to outnumber the rod-containing eosinophiles in the guinea hen, *Numida meleagris*, the kingfisher, *Alcedo atthis* (Cullen, 1903), and the duck, *Anas platyrhynchos* (Magath and Higgins, 1934).

The total number of leucocytes per cubic millimeter of blood is considerably higher in birds than in man, and the ratio of white to red cells may be from three and a half to ten times as high. There is some possibility, however, that avian white cells have sometimes been confused with hemolyzed red cells (Kyes, 1929).

Spindle-shaped thrombocytes are present in avian as in reptilian blood. These cells aid in thrombosis. There are no blood platelets, nor are megakaryocytes (thought to be the parent cells of platelets) found in the bone marrow of birds. The least numerous cells in the blood of birds are the plasma cells.

In adult birds, the formation of both red and white blood cells takes place predominantly in the bone marrow. In addition, the liver, spleen, and colic caeca produce both granular and nongranular leucocytes; in some species, erythropoiesis also occurs in these organs, but only for a limited time. The thymus and bursa of Fabricius, too, exhibit transitory hematopoietic activity. The blood-producing tissue in all of these organs is the so-called lymphoid or lymphatic tissue (Jordan, 1936).

The definitive hematopoietic organs are not present for some time after the start of incubation. During the largest portion of the embryonic period, the major locus of blood formation is the yolk sac, and erythropoiesis predominates over leucocytopoiesis. Nevertheless, both red and white blood cells begin to be produced within the embryonic body proper at a relatively early period. Gradually, hematopoietic centers become established. As the



chief hematopoietic organs start to function, the blood-forming activity of the yolk sac wanes. At hatching, however, the definitive blood picture has still not been attained.

Although there is general agreement as to the mesodermal origin of blood and vascular tissue, there is more than one school of thought regarding the lineage of red and white blood cells. According to the single-origin or monophyletic theory, all types of blood cells spring from a common ancestor derived in turn from a mesenchyme cell. Since red blood cells form intravascularly and white blood cells extravascularly, it is argued that environment determines the direction in which the primitive stem cells (or hemocytoblasts) differentiate. The monophyletic theory has been opposed by the concept of dual origin of the red and white cells, as well as by various multiple-origin (polyphyletic) theories. The dualistic and polyphyletic theories regard the mesenchyme cell as the only admissible common ancestor of red and white blood cells. The descent of all blood cells in both embryo and adult comprises a question that remains largely unsettled.

### Hematopoiesis in the Yolk Sac

The first embryonic blood cells are produced by the blood islands, which, as we have seen, are the aggregates of cells remaining within the lumina of the blood vessels forming in the yolk sac. The elements of the blood islands increase in number by mitosis, the division of all the cells being practically simultaneous (*Sabin, 1920*). It is only when cells division occurs that cell boundaries become visible in the blood islands (Fig. 224-A<sub>1</sub>, A<sub>2</sub>, and B), which at other times appear syncytial. In the chick embryo, the time interval between two divisions may be from 45 minutes to 3 hours and in general seems to be inversely related to the size of the blood island in which the cells are contained (*Muller, 1930*).

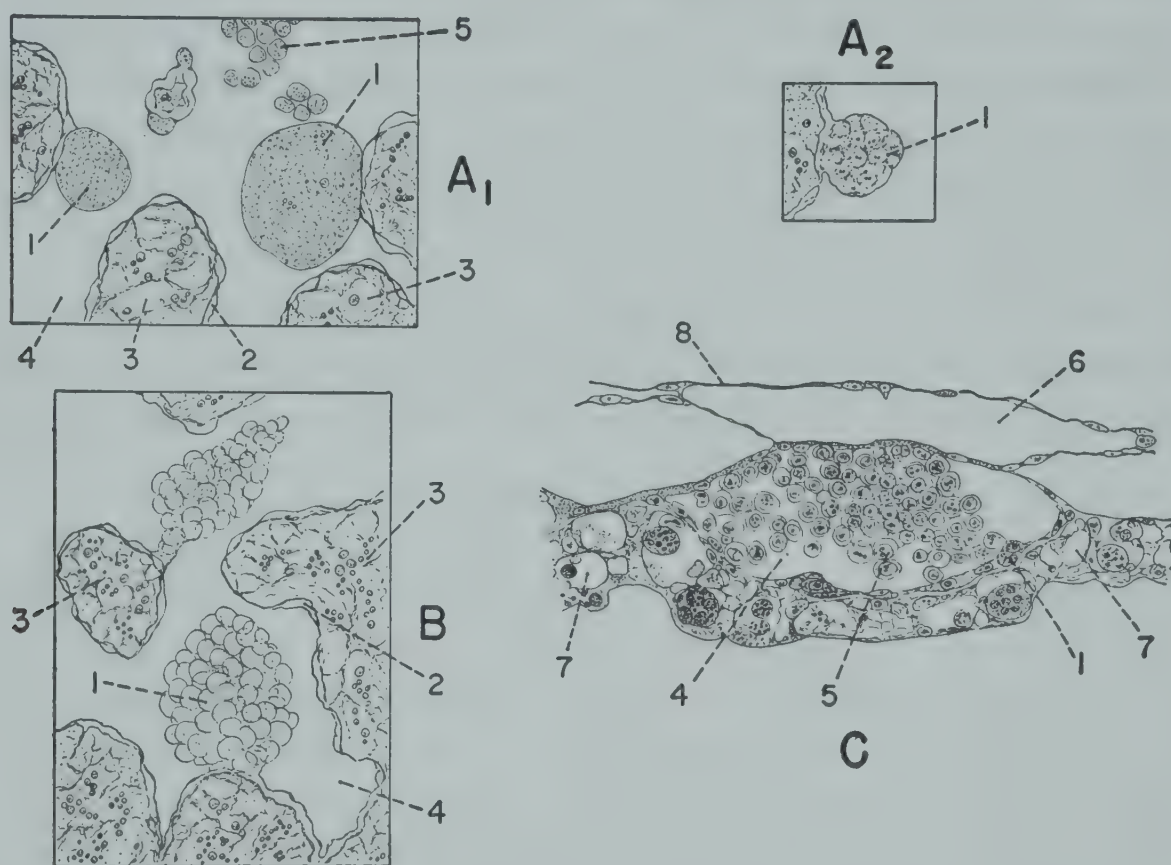
Almost immediately after the formation of blood plasma, hemoglobin becomes faintly visible in the blood islands (*Dehler, 1895; Sabin, 1920; Murray, 1932*). Wulf (1897) detected hemoglobin spectroscopically in 6-somite chick embryos, and Slonimski (1927*b*, 1931), by the use of a benzidine reaction (*Slonimski, 1927a*), demonstrated its presence in a narrow horseshoe-shaped zone outlining the posterior and lateral periphery of the area vasculosa of the 5- to 6-somite chick embryo and the 3- to 4-somite duck (*Anas platyrhynchos*) embryo. Other investigators, however, have not found hemoglobin until later, when there are from twelve somites (*Smiechowski, 1892*) to eighteen or twenty somites (*Danchakoff, 1907, 1908b*).

The blood islands gradually disintegrate into their individual elements (Fig. 224-A<sub>1</sub> and C). These are the first blood cells.

From the polyphyletic viewpoint, the first intravascular blood cells should all be considered as red cells (*Bizzozero, 1883, 1884; Stricht, 1892;*



*Janošik, 1902; Engel, 1915; Sabin, 1920, 1921; Slonimski, 1931*). The primitive intravascular blood cells have been called megaloblasts (*Sabin, 1921; Sugiyama, 1926*), and they are said to give rise only to erythroblasts (forerunners of erythrocytes). *Sabin (1920)* could find no white blood cells, or their early forms, in the area vasculosa of the 48-hour chick blastoderm, whereas red blood cells are circulating at this stage (*Féré, 1894a*). The extravascular mesenchyme cells are regarded as the ancestors of the white



**Fig. 224.** Blood islands in the 2-day chick embryo. (Redrawn with modifications after *Sabin, 1920*.)

$A_1$ , blood islands in the area pellucida as they appear in the living specimen;  $A_2$ , the phase of division of the nuclei of the blood island seen at the left in  $A_1$  ( $\times 200$ );  $B$ , blood islands taken from the posterior portion of the area pellucida; visible cell outlines indicate that the cells are undergoing division even though chromosomes do not show in living specimens ( $\times 200$ );  $C$ , section through the marginal sinus showing a new generation of blood islands beginning within that vessel ( $\times 300$ ).

1, blood island; 2, endothelium; 3, interspace between vessels as it appears in the living specimen; it represents the sub-vascular mesoderm not analyzed with reference to its cells and is bordered with a rim of endothelium; 4, lumen of the vessel or plexus of vessels; 5, free blood cells; 6, exocoelom; 7, endoderm of the yolk sac (ectoderm not shown); 8, mesoderm bordering the exocoelom.

blood cells. *Stricht (1892)* commented upon the resemblance between the granular, amoeboid mesenchyme elements and the granulocytes, and *Sugiyama (1926)* noted morphological dissimilarities between the extravascular “myeloblasts” and the intravascular “megaloblasts.”

According to the monophyletic theory, as stated especially well by *Danchakoff (1908a, 1908b)*, the primitive blood cells comprising the intact blood islands differentiate not only as erythroblasts but also as “large



lymphocytes." The large lymphocytes were regarded by Danchakoff as the common stem cells (equivalent to hemocytoblasts) of all blood cells subsequently formed. The stem cells give rise to additional erythroblasts, to further generations of stem cells, and (after migrating to an extravascular position) to granulocytes. Granulocytes thus have the same origin as erythrocytes, namely, the primitive cells of the blood islands.

Danchakoff (1907, 1908*a*, 1908*b*) believed, also, that some primitive blood cells remain isolated outside the newly formed blood vessels and are soon transformed into cells morphologically identical with hemocytoblasts. These extravascular cells increase in number not only by mitosis and by the addition of hemocytoblasts that emigrate from the blood vessels, but also by the proliferation of cells from the outer surface of the vascular endothelium.

Sabin (1921) corroborated the extravascular proliferation of cells from the endothelium on the chick's third day of incubation but identified the elements thus formed as clasmatoocytes (macrophages, or wandering phagocytic cells); Sugiyama (1926) agreed as to their classification but stated that they arise through the differentiation of mesenchymal cells peripheral to the endothelium, that is, in the adventitial position. Both these authors also found intravascular phagocytic cells of endothelial origin in the yolk sac of 2- to 3-day chick embryos, but Sabin (1922) classified them as monocytes and Sugiyama as macrophages.

Regardless of their origin, the first primitive white blood cells are more or less unanimously described as arising extravascularly in the vicinity of the yolk sac vessels on the chick's third or fourth incubation day (*Danchakoff*, 1908*b*; *Sabin*, 1921; *Sugiyama*, 1926). These cells give rise to eosinophiles with rod-shaped granules; the latter cells start to enter the blood stream on the fifth day (*Sugiyama*, 1926).

There is some possibility that the forerunners of thrombocytes may appear in the yolk sac vessels on the third or fourth day of incubation. Clumps of small, non-hemoglobin-containing cells were seen at this time by Sugiyama (1926) and identified as thromboblats. This author believed that thrombocytes had frequently been mistaken for lymphocytes by earlier observers. On the other hand, Fennell (1947) regarded these clumps of cells as being composed of hemocytoblasts and found no true thrombocytes until the eleventh day. The thrombocytes are probably closely related to the red blood cells (*Sugiyama*, 1926; *Jordan*, 1936; *Fennell*, 1947).

On the chick's fourth day of incubation, the blood-forming activity of the yolk sac is intensified. The number of hemocytoblasts starts to increase preparatory to the production of a new strain of erythroblasts, the definitive series. Hematopoiesis in the yolk sac is at its peak between the tenth and the fourteenth or fifteenth days (*Danchakoff*, 1908*b*) and then de-



clines. It gradually comes to an end during the period between the eighteenth and the twentieth day.

*Experimental modification of extraembryonic hematopoiesis.* The injection of one or more sources of vitamin A or D into the 24-hour incubated chicken egg apparently creates a vitamin imbalance that is manifested in various ways 24 to 48 hours later (*Hansborough, 1947a*). Among other effects, there is a retardation in blood and vessel formation, as indicated by the presence of abnormally large hemangioblasts and by deficiencies in hemoglobin or the number of erythrocytes, or both. There may be clots in the sinus terminalis and other vitelline vessels. Similarly, the injection of 20 mg. of nicotinic acid into the egg prior to incubation adversely affects hemoglobin and erythrocyte formation (*Hansborough, 1947b*).

Jeney and Törö (1935) injected various substances into chicken eggs incubated 40 hours and observed the effects 46 hours later. Hematopoiesis was encouraged in all cases by the injection of homologous hemoglobin, globin solution, liver extracts, arginine, and valine, and in some cases by the injection of the saliva of healthy people.

### Intraembryonic Hematopoiesis

The decrease in the blood-forming activity of the yolk sac at the beginning of the chick's third week of incubation follows the initiation of hematopoiesis in organs situated within the embryo. In certain intraembryonic blood-forming centers the production of some types of blood cells ceases before the hatching date; in others, blood cell formation continues indefinitely (Fig. 225; Fig. 226). The bone marrow, the chief definitive focus of both red and white blood cell formation, is the last in which hematopoiesis begins. There is disagreement regarding the significance of the liver and spleen as blood-producing organs, but the weight of the evidence seems to indicate that they play a role in the formation of white blood cells in both the embryonic and the adult chicken. In passerine birds, as exemplified by the starling (*Sturnus vulgaris*), intraembryonic hematopoiesis is initiated relatively later in the incubation period than it is in the chick (cf. Fig. 225), and the liver and spleen actively participate in erythropoiesis before hatching and for a few weeks afterward (*Sandreuter, 1951*).

Long before intensive blood formation occurs in the major hematopoietic organs, a few blood cells are produced intraembryonically; in fact, hematopoiesis starts within the embryo not long after it begins in the yolk sac. The presence of blood islands has been noted within the dorsal aorta at the level of the most caudal somites of the 8- to 9-somite chick embryo (*Sabin, 1920*). Several investigators have reported that, on the chick's third or fourth incubation day, the endothelium of the ventral wall of the entire aorta begins an intensive intravascular proliferation of hemocytoblasts,



which, like cells in the intravascular position in the yolk sac, give rise to erythrocytes (Danchakoff, 1907, 1909a; Jordan, 1917; Sabin, 1917a). The proliferation of cells is said to cease gradually on the sixth and seventh days. The same process has been seen in other vessels (Stricht, 1892), such as the superior mesenteric artery (Jordan, 1917). The endothelium of the heart and the aortic arches has also been observed to proliferate a few isolated clumps of cells (Danchakoff, 1909a). Danchakoff (1909a) concluded that

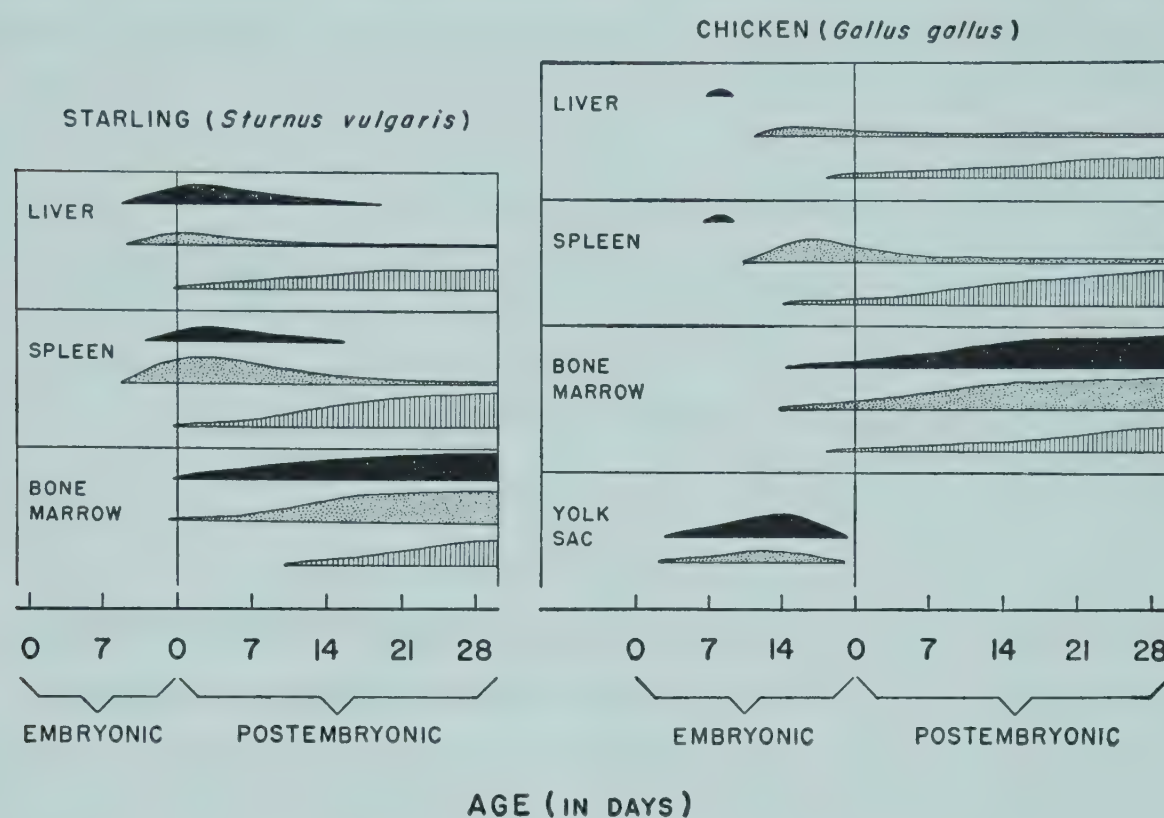


Fig. 225. Schematic representation of the chronology of hematopoiesis in the liver, spleen, bone marrow, and yolk sac of the chick during the embryonic and early post-embryonic period, as compared with the same process in the starling, *Sturnus vulgaris*. (Redrawn with modifications after Sandreuter, 1951.)

Erythropoiesis is represented by the solid black field, granulocytopoiesis by the stippled field, and lymphopoiesis by the vertically hatched field. The intensity of hematopoiesis is indicated by the width of the fields.

all embryonic vascular endothelium possesses hematopoietic potency, which is exhibited to a different extent in different locations. Jolly (1939–40), on the other hand, failed to find any intravascular proliferation of red blood cells after the vessels become closed and circulation begins, and he denied that such proliferation occurs.

Blood is also said to be formed in the loose mesenchymal tissue. Prior to the chick's fourth day of incubation, blood islands, similar to those found in the yolk sac, differentiate in the head mesenchyme. According to Danchakoff (1909a), these blood islands give rise to cells that contain hemoglobin but are destined to degenerate or to be phagocytized; Sabin (1920), however, believed that the presence of any red blood cells in the extravascular tissues is due to extravasation resulting from trauma.

On the fourth and fifth days, wandering cells begin to differentiate in



the head mesenchyme, in the loose mesenchyme dorsal to the somites, and in the mesentery ventral to the aorta. These wandering cells are of two varieties, the typical hemocytoblast and the macrophage. The differentiation of hemocytoblasts from mesenchyme soon ceases (*Danchakoff, 1909a*). The extravascular hemocytoblasts form foci of hematopoietic tissue in certain localized spots. Accumulations of these cells are found around the heart and the aorta, in the tissues dorsal to the pharynx, around the cranial nerves and ganglia and the spinal ganglia, between the spine and the esophagus, and in the subcutaneous tissues and muscles (*Danchakoff, 1909a*).

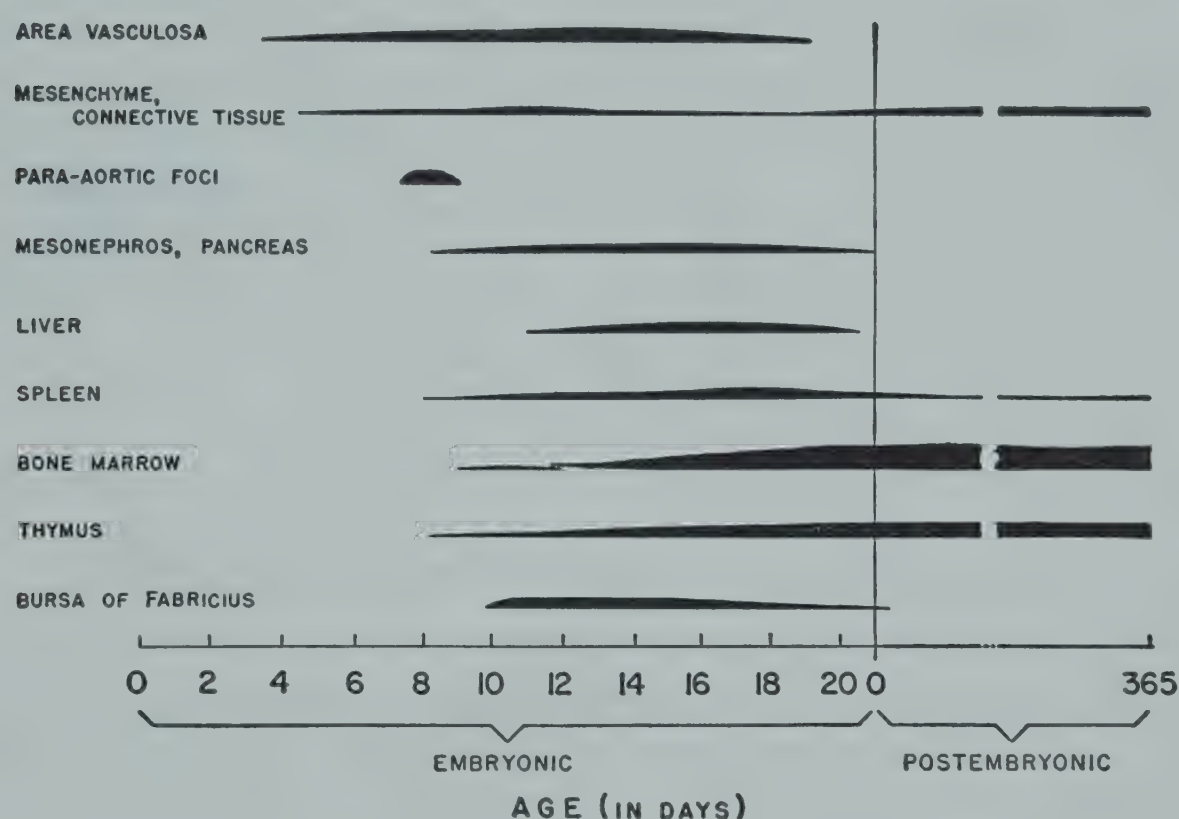


Fig. 226. Diagram showing the location, intensity (represented by the width of the black lines), and the chronology of eosinophilic leucocytopoiesis in the chick before and after hatching. (Redrawn with modifications after Terni, 1924b).

As in the yolk sac, the first white blood cells to differentiate extravascularly are the immature eosinophilic granulocytes. Terni (1924b) remarked that the period of localization of granulocytopoietic centers in the young connective tissue is followed by a period during which this tissue invests or insinuates itself into various organs of endodermal origin (liver, thymus, and bursa of Fabricius) or of mesodermal origin (mesonephros, spleen, bone marrow). These are the organs which contain the hematopoietic lymphoid tissue.

According to Danchakoff (1909a), the extravascular hemocytoblasts start to produce "small lymphocytes" on the chick's tenth or twelfth day of incubation. These cells first appear in loose connective tissue and are distinguished from hemocytoblasts chiefly by the reduction in size of both cytoplasm and nucleus. They supposedly give rise to macrophages, plasma



cells, and granulocytes. In adult birds, small lymphocytes, produced in the lymphoid nodules of hematopoietic tissue, have been regarded as representing the intermediate stage between hemocytoblasts and both erythrocytes and granulocytes (*Jordan, 1936*).

### *Blood Formation in the Liver*

The liver is far less significant as an embryonic hematopoietic organ in birds than in mammals (*Prévost and Dumas, 1824; Bizzozzero, 1883; Stricht, 1891*). Blood formation was observed in the sinusoids of the liver by Stricht (1891), but new red blood cells formed there were thought to arise through the division of pre-existing erythroblasts. Haff (1914), however, claimed that there are two distinct embryonic periods during which blood is formed in the liver of the chick. The first begins on the seventh day of incubation. Marked hematopoietic activity is exhibited by a loose, perivascular reticulum that develops from the endothelium of the closed capillaries and from the peritoneal cells covering the liver. The connective tissue cells of this reticulum differentiate to erythrocytes and granulocytes, although granulocytopoiesis is very slight. At the end of the ninth day, the capillary endothelium and the peritoneum return to their original state. On the eleventh day, intensive granulocytopoiesis begins in the once more actively proliferating perivascular connective tissue. This process reaches its maximum on the fourteenth or fifteenth day and gradually ceases before the end of incubation. Haff's observations were confirmed by Sandreuter (1951), who noted, in addition, that immature lymphocytes and monocytes and the various developmental forms of eosinophilic (amorphous granular) and basophilic granulocytes are present in the liver on the eighteenth day of incubation. This author also reported that erythropoiesis and granulocytopoiesis begin simultaneously in the liver of the starling (*Sturnus vulgaris*) embryo on the eighth incubation day. Immature lymphocytes first appear on the twelfth or thirteenth day (that is, directly before hatching) when the immature forms of all types of granulocytes are seen. Red blood cell formation is most intensive during the first 5 post-hatching days and gradually ceases between the tenth and the twentieth days. Granulocytopoiesis is reduced after the fifth post-hatching day, and lymphoid elements, including monocytes, increase after the tenth day.

Danchakoff and Sharp (1917) suggested that fetal hematopoiesis is more intense in the mammalian liver than in the sauropsidan liver because the blood reaching the liver sinusoids in mammals comes through the umbilical veins from the placenta and is rich in the specific nutrients necessary for erythrogranulocytopoiesis. In sauropsidans, the blood carried to the liver has passed through the venous plexus of the yolk sac and may have been filtered of the substances necessary for the differentiation of mesenchyme to blood-forming tissue.



### *Blood Formation in the Thymus*

In the chick embryo the thymus is second only to the bone marrow in leucocytopoietic activity (*Terni, 1924b*). On the eighth day of incubation, a few eosinophilic granulocytes can be seen in the inner portion of the anlage of the thymus; on its periphery, there is an accumulation of hemocytoblasts that possibly have migrated from other leucocytopoietic centers, such as the para-aortic focus. A day or two later, these hemocytoblasts begin their transformation into eosinophilic granulocytes, and the immature cells produced in this way start to divide on the eleventh day. Their granules assume a gradually more elongated form with the passage of time (*Terni, 1924b*). Meantime (before the tenth day), small lymphocytes have begun to differentiate from extravascular hemocytoblasts, and various transitional forms are present. The thymus is thus the first functioning lymphoid organ to be established. Small lymphocytes formed here enter the blood vessels and are carried to other portions of the body, where they leave the capillaries and again enter the tissues (*Danchakoff, 1909a*). Erythropoiesis also occurs in the thymus, but only to a very slight extent (*Terni, 1924b*).

### *Blood Formation in the Spleen*

On the chick's eighth or tenth day of incubation (*Jolly, 1911; Danchakoff, 1916; Sandreuter, 1951*), many hemocytoblasts are already present in the loose mesenchymal syncytium of the spleen, and erythroblasts may be seen within the forming venous sinuses. Erythroblasts are not found in large numbers, however, and the spleen is not considered an active erythropoietic organ in the chick (*Danchakoff, 1916; Warren and Dixon, 1949b*). It probably functions as such for only a few days, possibly 5 (*Sandreuter, 1951*). In the starling (*Sturnus vulgaris*), however, pronounced erythropoiesis occurs in the spleen. The process begins on the eighth incubation day, reaches its maximum a few days after hatching, and continues until the twentieth post-hatching day (*Sandreuter, 1951*).

By the chick's eleventh day (*Danchakoff, 1916*) or twelfth day (*Jolly, 1911*) the hemocytoblasts in the vicinity of the venous sinuses begin to differentiate as eosinophilic granulocytes. Granulocytopoiesis is intense, continuing until the end of the incubation (*Danchakoff, 1916*), but reaching a maximum on the sixteenth to seventeenth days (*Jolly, 1911*). Eosinophiles are present in the spleen of the starling (*Sturnus vulgaris*) on the eighth day (*Sandreuter, 1951*), but their maximum production is attained after hatching.

On the chick's twelfth or thirteenth incubation day, intensive arterial vascularization of the spleen begins, and the white pulp appears as islands of mesenchymal tissue around the arterial vessels. In the peripheral por-



tions of the islands, numerous hemocytoblasts of small size proliferate; beginning on the fourteenth or fifteenth day, these cells differentiate into small lymphocytes (Danchakoff, 1916; Sandreuter, 1951). Sandreuter (1951) noted that monocyte forms also increase as the small lymphocytes become more numerous. This investigator found that lymphocytes appear in the starling (*Sturnus vulgaris*) embryo's spleen on the twelfth day. In both the chick and the starling, their production increases after hatching.

Basophilic and pseudoeosinophilic (amorphous granular) leucocytes are also formed in the spleen of the chick and the starling (*Sturnus vulgaris*) during the late embryonic period (Sandreuter, 1951).

Sugiyama (1926) stated that the endothelium of the venous sinuses of the spleen produces macrophages intravascularly until postembryonic life.

Danchakoff (1924a) observed that chorioallantoic grafts of the chick spleen anlage do not develop embryonic leucocytes.

#### *Blood Formation in the Bursa of Fabricius*

Beginning between the chick's tenth and twelfth incubation days, hemocytoblasts differentiate from mesenchyme cells surrounding the lymphoepithelial buds in the internal portion of the forming bursa of Fabricius. These cells go through all the maturation phases leading to the formation of eosinophilic leucocytes. During the last days of incubation, when hematopoiesis in the bursa is at its peak, masses of young red blood cells are seen in the mesenchyme. Neighboring mesenchymal cells have been said to group themselves around the red blood cells as if forming blood vessels. Both erythropoiesis and granulopoiesis in the bursa cease within a few days after hatching. These processes have been observed in the bursa of the duck (*Anas platyrhynchos*), pigeon (*Columba livia*), and goshawk (*Accipiter gentilis*), as well as in that of the chick (Jolly, 1915).

#### *Blood Formation in the Bone Marrow*

The long bones and the vertebral bodies contain the largest proportion of blood-forming marrow, which, however, is found to some extent in all bones. Hematopoietic activity in the marrow of the long bones begins in the chick on the eighth or ninth day of incubation. During a transitory phase of blood formation lasting from the ninth to the twelfth day, some of the extravascular mesenchymal cells are transformed *in situ* into "small lymphocytes" which give rise to eosinophilic leucocytes, but the latter degenerate or are phagocytized (Danchakoff, 1909b). The definitive hematopoietic process starts on the tenth or twelfth day of incubation (Danchakoff, 1909b; Dawson, 1936; Warren and Dixon, 1949b) with the differentiation of typical hemocytoblasts from extravascular mesenchyme cells.



Some hemocytoblasts enter the vessels and, lining the venous sinusoids, give rise to red blood cells and thrombocytes; others remain in the extravascular position and produce eosinophilic leucocytes. Early developmental forms of erythrocytes and granulocytes are visible on the fourteenth day. At this time, there also appear basophilic forms which, according to Danchakoff (1909a, 1909b), are derived from the small lymphocytes of the marrow reticulum. The presence of pseudoeosinophilic (amorphous granular) leucocytes at this time was noted by Sandreuter (1951), who observed immature monocytes and lymphocytes on the eighteenth day and remarked that erythropoiesis is markedly increased during the last 3 days of development. A slight increase in the percentage of mature erythrocytes also occurs during this period (Burmester, Severens, and Roberts, 1941). In the starling (*Sturnus vulgaris*), hematopoiesis begins in the bone marrow on the day before hatching (that is, the twelfth incubation day).

The blood cells develop within the marrow until they are mature, when the erythrocytes are released into the blood stream and the white blood cells migrate into the circulation (Danchakoff, 1909b). In the adult chicken, the forerunners of the red blood cells allegedly are included within the venous sinuses of the bone marrow when reticular cells are transformed into endothelial cells and are incorporated into the walls of the venous sinuses (Jordan, 1936).

*The effects of X-rays on hematopoiesis in bone marrow.* Lethal or sublethal continuous radiation has been applied to the bone marrow of the chick embryo by injecting an isotope of phosphorus,  $P^{32}$  (as  $KH_2PO_4$ ), into incubating eggs (Warren and Dixon, 1949b). Sublethal radiation (from injection of 120  $\mu c$  on the fourth day or of 100  $\mu c$  on the eighth or the fourteenth day) results in reduction of mitotic activity and in inhibition of the maturation of both red and white blood cells. The effects are most pronounced in the well-calcified areas. The proportion of immature forms increases noticeably between the fourth and seventh days after injection and then remains constant. Mitotic activity continues for a few days after injection and then decreases as the radiation injury becomes more extensive. During the second week after injection, the number of marrow cells is reduced to one fourth or one third of the normal number, and most of them are immature. Two or three weeks after injection, the immature cells resume mitotic activity and soon afterward continue their maturation. Complete recovery usually follows, although small foci of undifferentiated lymphoid cells are occasionally seen in the marrow of injected birds. The reticuloendothelial cells, although somewhat enlarged, are otherwise little affected by radioactive phosphorus and are able to carry on a significant amount of hematopoiesis after radiation has been applied.

Lethal continuous radiation (180 and 200  $\mu c$ ) causes all maturation and mitotic activity to cease within 2 or 3 days after injection. Two weeks after



injection, injury to the bone marrow reaches its maximum. Scattered regeneration may occur subsequently, but it is insufficient to compensate for the destroyed tissue, the embryo succumbing to a fatal pancytopenia (loss of all blood cells).

### *Blood Formation in Other Organs*

In the middle of the eighth day of incubation, additional foci of granulocytopoiesis (and sometimes of erythropoiesis, also) appear in various organs of the chick embryo. They are found in the mesenchyme around the mesonephros and in the interstitial connective tissue of that organ, especially in the vascular glomeruli of the more cranial portion. According to Chishima (1952) the deficiency of fixation and differentiation of erythrocytes in the right ovary is the chief factor in the retrogression of that organ.

Eosinophilic granulocytes accumulate in groups in the primordium of the pancreas. After the ninth day, similar foci are present in the vicinity of the gonads, in the metanephros, and in the renal capsule, always in proximity to vessels (Nonidez, 1920).

### **The Primitive and the Definitive Red Blood Cells**

It is fairly definite that the first red blood cells to be formed constitute a primitive strain that is destined to degenerate and to be replaced by a definitive strain (Boccardi, 1886; Engel, 1895, 1915; Danchakoff, 1908b; Dawson, 1936). The primitive cells dominate the blood picture from the chick's second to fourth days of incubation. On the third or fourth day, the presence of erythroplastids (Dawson, 1936) and microcytes (Danchakoff, 1908b)—representing, respectively, the cytoplasmic and nuclear remnants of cells—indicates that some elements have begun to degenerate. Despite the early onset of degeneration, a few cells of the primitive strain may persist until 2 weeks after hatching (Dawson, 1936), but Lemez and Rychter (1956) observed the maximal life span of primitive erythrocytes to be 22 days with none found on the chick's fourth postembryonic day. At some time between the fourth day (Dawson, 1936) and the sixth day (Fennell, 1947), the first cells of the definitive strain appear.

In both strains, comparable types of immature cells are formed before the ultimate cell, the erythrocyte (primitive or definitive), is produced. Distinction between the successive immature types of either strain is usually made on the basis of the shape of the cell, the hemoglobin content, the appearance of the cytoplasmic organelles, and the various staining properties (Fig. 227). Bernardelli, Rondanelli, and Gorini (1953) found that the diameters of cells and nuclei of embryonic chick erythroblasts decrease as differentiation increases. The cytoplasm diameter remains practically constant after the basophilic stage. An important phenomenon char-



acterizing the progressive maturation of the red cells is the decreasing basophilia of the cytoplasm and its increasing eosinophilia, changes which are correlated with a rise in hemoglobin content.

The first elements of the primitive strain are the large slightly amoeboid cells derived from the blood islands and probably containing a small amount of hemoglobin (*Sugiyama, 1926*). These cells give rise to two successive "generations," or types, of primitive erythroblasts, which are increasingly oval and flat (cf. Fig. 227), and in which the amount of hemo-

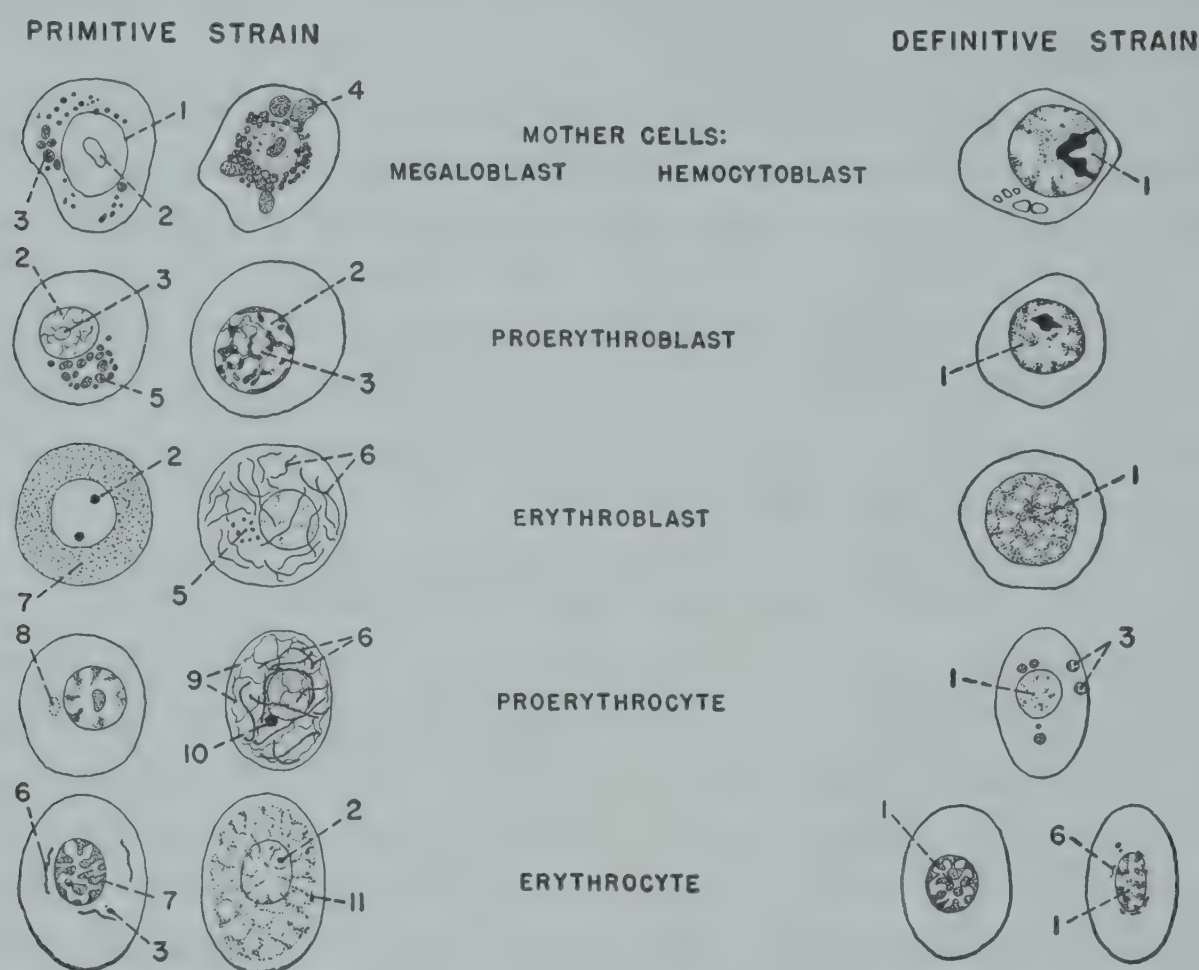


Fig. 227. Various successive forms in the differentiation of primitive and definitive red blood cells in the developing chick embryo. (Reconstructed after *Danchakoff, 1909a*; *Sugiyama, 1926*; *Dawson, 1936*; and others.)

Cells of the primitive strain (left side) at successive stages, as they appear when stained by different methods to show their various component parts. Comparable differentiating forms of the definitive strain are shown on the right side. All  $\times 1000$ .

1, nucleus; 2, nucleolus; 3, neutral red granules; 4, precipitated basophilic substance; 5, rosette of neutral red granules; 6, filamentous mitochondria; 7, basophilic granules; 8, "hof"; 9, fine reticulum; 10, Golgi apparatus; 11, basophilic reticulum.

globin is progressively augmented. Round primitive erythroblasts, type I (often known as primitive proerythroblasts), predominate on the chick's second incubation day. They are largely replaced 24 hours later by erythroblasts, type II, which, though also round, are lenticular in profile and have an eccentric nucleus (*Dawson, 1936*). The slightly oval, immature primitive erythrocytes, frequently termed primitive proerythrocytes (cf. Fig. 227), may be present in large numbers before the fourth day (*Dawson, 1936*), but the first mature primitive erythrocytes (cf. Fig. 227)



appear between the fifth and seventh days (*Dawson, 1936; Fennell, 1947*). According to Dawson (1936), the mature primitive erythrocytes are briefly predominant at the beginning of the sixth incubation day, but this is probably the only time when they are not greatly outnumbered.

In cultures of explants from the area opaca, primitive erythroblasts have been seen to differentiate from small, mononuclear, basophilic cells. These erythroblasts formed *in vitro* contained fragmented nuclei and were not observed to give rise to erythrocytes (*Shipley, 1916*).

The maturation of the chick embryo's primitive red blood cells is accelerated somewhat when folic acid or liver extract is injected into the egg prior to incubation (*Perri, 1948a; Riggio, 1942*). Treatment with these substances results in the appearance of large numbers of primitive proerythrocytes in the chick embryo's blood after 60 hours' incubation, or about 12 hours in advance of the normal time. The dosage of folic acid influences the relative number of proerythrocytes produced, as the accompanying data indicate (*Perri, 1948b*). A slight acceleration of maturation

Folic Acid Injected (micrograms)	Proportion of Proerythrocytes (per cent of total erythrocytes)
1	15
5	27
15	48
20	58
30	61
40	68
50	71

also occurs *in vitro* when cultures of red cells are treated with liver extract or folic acid. Megaloblasts of chick embryos cultivated *in vitro* with hepatic extract or folic acid after 60 hours' incubation showed reduction in size compared with controls not receiving the extract (*Perri, Borghese, and Gelmetti, 1951b, 1953*). Since mitosis is not stimulated by these substances, the effect may be deemed a true promotion of differentiation.

The cells of the definitive strain (cf. Fig. 227) are readily distinguishable from those of the primitive strain. In general, they are smaller, less granular, and more oval than their primitive counterparts, and their oval shape is acquired more quickly. Also, their hemoglobin content is lower (*Dawson, 1936*). Lemez (1953) found in the embryonic chick that definitive erythrocytes with round nuclei are replaced step by step by those having oblong nuclei, these constituting on the seventh day 1 per cent of all erythrocytes and 97.9 per cent on the eighteenth day.

It appears most probable that the cells of the definitive strain are derived in major part from the intravascular, basophilic hemocytoblasts (*Danchakoff, 1908b*), which may or may not (according to the different



TABLE 9

The Percentages of the Various Forms of Red Cells Found in the Blood of the Chick Embryo on Successive Incubation Days \*

Percentages of Various Red Cell Types											
Primitive Strain						Definitive Strain					
Age of Chick Embryo	(days)	(hours)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)
			Megaloblasts	Proerythroblasts	Erythroblasts	Proerythrocytes	Erythrocytes	Hemocytoblasts	Proerythroblasts	Erythroblasts	Proerythrocytes
											Erythrocytes
2	2	0	29.6	70.4							
2	2	16	0.2	4.2	95.4						
3	3	0	0.2	0.2	14.0	85.4					
3	3	23			3.0	93.1	0.7	2.0	0.6		
4	4	16				98.9		0.6	0.4		
4	4	22				98.8		0.4	0.6		
5	5	3				9.9	79.5	0.7	2.4	6.9	
5	5	20					23.9	0.4	0.3	74.9	
6	6	3					20.3		1.7	36.3	40.6
7	7	0					11.7			7.6	80.2
8	8	0					10.3			0.2	89.5
9	9	0					5.4	0.2		0.9	93.6
10	10	0					6.6	0.1		0.4	92.5
11	11	0					3.7				96.2
12	12	0					3.8	0.6		0.4	95.2
13	13	0					4.8		0.1	0.5	10.4
14	14	0					3.8		0.4	4.2	17.1
15	15	0					2.1			0.2	26.9
16	16	0					0.5			0.5	6.0
17	17	0					0.6				1.9
18	18	0					0.2			0.3	0.9
											84.2
											74.5
											70.7
											92.6
											97.4
											98.4

\* After Dawson (1936).



theories of blood formation) be identical with the ancestors of the white blood cells. It is of passing interest to note that Sugiyama (1926) favored the primitive erythroblasts as the direct forebears of the definitive, and that Engel (1915) denied the descent of the definitive erythrocytes from the definitive erythroblasts, or metrocytes of the second generation, in his terminology.

Definitive erythroblasts, type I (that is, definitive proerythroblasts), have basophilic cytoplasm and are produced on the chick's fourth to seventh incubation days (*Danchakoff, 1908b; Dawson, 1936*). At the end of the sixth day, definitive erythroblasts (or definitive erythroblasts II) suddenly become the predominating forms (*Dawson, 1936*). Erythroblasts with basophilic and with polychromatic cytoplasm are both present, the polychromatic cells being more mature and containing more hemoglobin (*Sandreuter, 1951*). Definitive proerythrocytes, also polychromatic, are the most numerous red cells on the seventh day (*Dawson, 1936*) and are in turn outnumbered by mature definitive erythrocytes on the tenth day (*Sandreuter, 1951*) or thirteenth day (*Dawson, 1936*). A slight rise in the number of proerythrocytes on the fourteenth day (*Dawson, 1936*) coincides with the peak in the hematopoietic function of the yolk sac (*Sandreuter, 1951*). Proerythrocytes virtually disappear by the hatching date, and the mature erythrocytes increase to 98 or 99 per cent of the red cells (*Dawson, 1936; Fennell, 1947; Sandreuter, 1951*).

Incubation at elevated atmospheric pressure retards the maturation of the chick embryo's definitive red cells. In 10-day embryos incubated under a pressure of 40 pounds, the red cells appear less mature than those of normally incubated 8-day embryos (*Flemister and Cunningham, 1940*). The injection of liver extract into the egg prior to incubation appears to have no consistent effect on the formation or maturation of the chick's definitive red cells in the yolk sac but leads to their earlier release into the circulation (*Hays, Last, and Koch, 1942*).

The actual percentages of the various primitive and definitive red cells present in the chick's circulation during incubation may be found in Table 9. Both in this table and in Fig. 228, it is clear that the red cells of one stage disappear and are succeeded by those of the next stage with distinct regularity.

It is of interest to note that a recrudescence in the percentage of definitive proerythrocytes in the chick's blood stream occurs soon after hatching, probably because of increased hematopoiesis in the bone marrow (*Sandreuter, 1951*). They attain their maximum number (14 per cent of the total red cells) on the fifth post-hatching day, and are present in considerable proportion for the first month of postembryonic life. Erythroblasts are not seen in the blood stream after the eighth postembryonic day.



In the starling (*Sturnus vulgaris*), the definitive erythrocytes appear on the eighth incubation day and mature more quickly than in the chick; by the time the bird hatches, 5 days later, the definitive erythrocytes dominate the blood picture exactly as they do in the chick. The primitive erythrocytes disappear more quickly and are no longer found 3 days after hatching. The starling also differs from the chick in that the post-hatching increase in immature cells is earlier and more intensive. It reaches its maximum on the third post-hatching day, when erythroblasts and proerythrocytes may constitute 7 and 52 per cent, respectively, of the total red blood cells. This

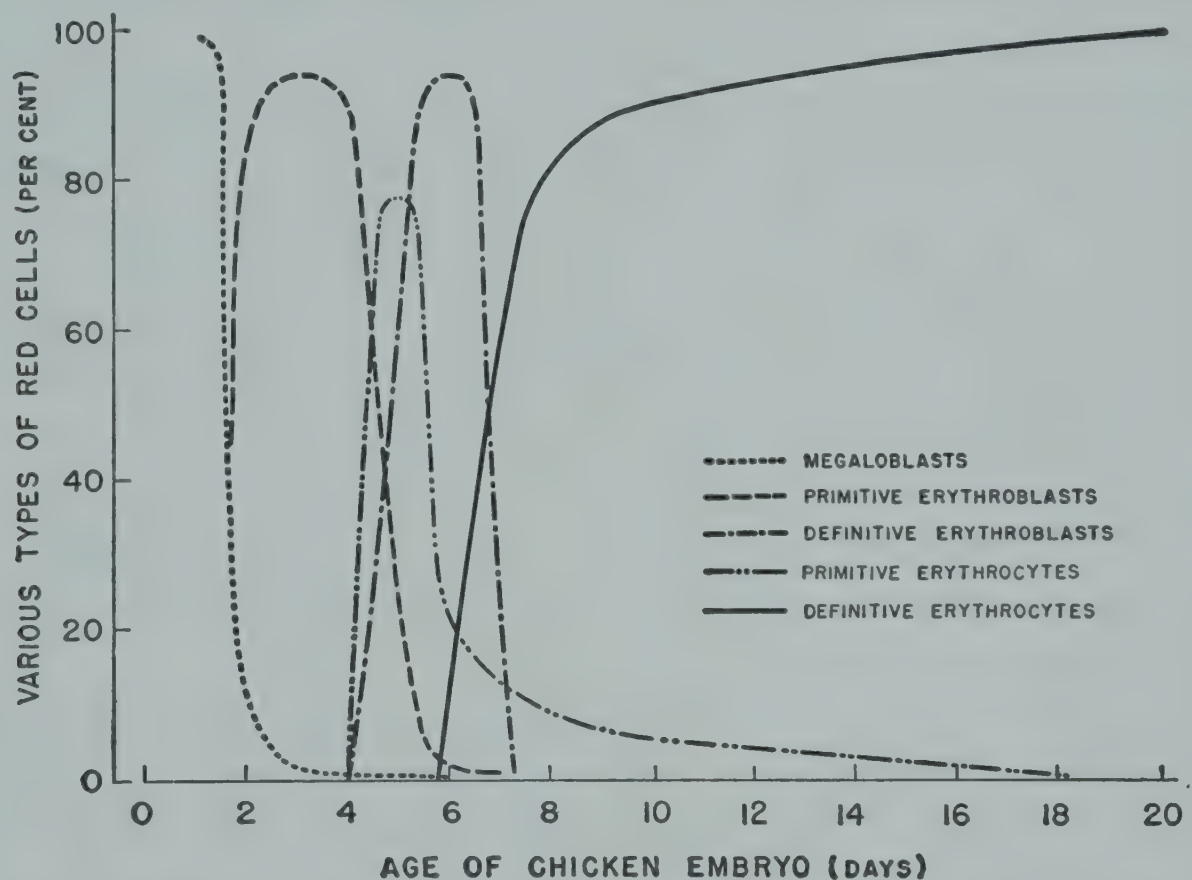


Fig. 228. Distribution of various types of red cells in the peripheral blood of the chick embryo during successive stages of development. (Megaloblasts, primitive and definitive erythroblasts, drawn after the data of Sugiyama, 1926; primitive erythrocytes, after Dawson, 1936; definitive erythrocytes, after Sugiyama, 1926; Dawson, 1936; Fennel, 1947.)

maximum coincides with the peak in hematopoiesis in the liver and spleen. Erythroblasts are found for nearly a month after hatching, and proerythrocytes are present during the entire first year of life (Sandreuter, 1951).

#### *Mitotic Activity of Embryonic Red Blood Cells*

The red blood cells are apparently the only elements of the embryonic blood that undergo mitosis while circulating in the vessels. Dawson (1936) found no dividing thromboblats, thrombocytes, or white blood cells in the blood stream.

As the accompanying data show (Dawson, 1936), the cells of the primitive strain (especially the primitive proerythrocytes) are more active mitotically than the cells of the definitive strain, because many of the latter



are retained in the hematopoietic centers until they are at the proerythrocyte stage. The number of dividing red cells in the circulating blood of the chick embryo increases from the second or third day to the fifth or sixth day (*Bizzozero*, 1883, 1884; *Dawson*, 1936) and then decreases (*O'Connor*, 1952*b*) until, on and after the eighth or ninth day, mitotic figures are rarely if ever seen (cf. Fig. 230).

Incubation Age of Chick	Average Mitotic Activity	
	Primitive Strain (per cent)	Definitive Strain (per cent)
48 hr.	1.49	
64 hr.	2.34	
72 hr.	3.44	
3 days 23 hr.	3.78	
4 days 16 hr.	3.24	
4 days 22 hr.	2.22	
5 days 3 hr.	0.70	
5 days 20 hr.		2.22
6 days 3 hr.		0.70
7 days		0.56
8 days		0.06
9-20 days		Occasional

*Astaldi*, *Bernardelli*, and *Rondanelli* (1951) concluded as a result of investigations of embryonic chick blood cells of the first 9 days of incubation that considerable proliferative activity takes place in early stages of their development. However, as differentiation of cells becomes more advanced, proliferative activity decreases and finally ceases altogether.

The mitotic activity (*in vitro*) of the red cells of the primitive strain is not increased by liver extract (*Muller*, 1930) or folic acid (*Perri*, *Borghese*, and *Gelmetti*, 1951*c*).

Investigations on 3-hour chick embryo blood islands and on 60-hour chick circulating blood cells to determine the rhythm of the proliferating primitive erythroblast show such proliferation is not synchronous but is governed by the law of fluctuating variability (*Astaldi*, *Bernardelli*, and *Rondanelli*, 1953).

### *The Size of Embryonic Red Blood Cells*

In the embryo, the red blood cells of both the primitive and the definitive strain are larger than the erythrocytes found in the blood stream of the adult bird. Throughout incubation, there is a progressive diminution in the size of the red cells.

The round primitive proerythroblasts found in the chick during the second or third day of incubation average about 14 microns in diameter (*Perri*, 1948*c*) but may measure as much as 16.5 microns (*Perri*, 1948*a*, 1948*c*). The diameter of the primitive erythroblasts formed thereafter may



range from 13 microns down to 8 microns (*Fennell, 1947*), although atypical, giant cells with two to four nuclei are often present during the first week of incubation (*Dawson, 1936*). Primitive erythrocytes measure about 8.95 by 12.7 microns (*Fennell, 1947*). The proerythroblasts of the definitive strain are from 10 to 12 microns in diameter (*Sandreuter, 1951*). The erythroblasts range from 8 to 10 microns in diameter, and the definitive erythrocytes average 7 by 10.3 microns (*Fennell, 1947*), although definitive erythrocytes of somewhat larger size (7 to 10 microns by 12 to 15 microns) have been reported in the chick at hatching (*Sandreuter, 1951*).

A decline in the average size of the definitive erythrocytes has been found to occur after hatching in the pigeon, *Columba livia*, the gull, *Larus* sp. (*Kalabukhov and Rodionov, 1934*), the chicken, and the starling, *Sturnus vulgaris* (*Sandreuter, 1951*). In the chicken, the postembryonic decrease in the average size of the erythrocytes is pronounced and takes place on the second post-hatching day, when cells of adult size arrive in the blood stream. The larger cells persist, however, for 6 to 10 weeks, although gradually dying out (*Lange, 1919; Sandreuter, 1951*). A second decrease in average red cell size becomes apparent when the large cells are no longer present.

In the starling (*Sturnus vulgaris*) embryo, the primitive and definitive erythrocytes are larger than those of the chick embryo. The definitive erythrocytes of adult size are not seen until the sixteenth post-hatching day, but the large cells disappear before the twenty-third day (*Sandreuter, 1951*).

The erythrocytes of the adult chicken, starling (*Sturnus vulgaris*), and pigeon (*Columba livia*) are very similar in appearance. The cells are oval, and their dimensions vary within the same limits: 5.5 to 7.5 microns by 11 to 14 microns (*Klieneberger and Carl, 1912; Lange, 1919; Perri, 1948b; Perri and Bo, 1948; Sandreuter, 1951*).

Red blood cells that develop in tissue explants taken from the area opaca prior to the appearance of blood islands are generally much smaller than those that develop *in vivo* (*Shipley, 1916*). Red blood cells from the 60-hour chick embryo, however, may be successfully maintained *in vitro* for 24 hours without alteration in size (*Perri, Borghese, and Gelmetti, 1951a*).

### *The Number of Definitive Erythrocytes*

In the chick embryo, there are about 1,500,000 definitive erythrocytes per cubic millimeter of blood on the twelfth day of incubation (*Ascarelli, 1895; Penionschkevitch, 1937; Ogorodniy, 1939; Sandreuter, 1951*). Thereafter, there is a general but rather irregular increase in the erythrocyte count (Fig. 229), except, perhaps, for a decrease between the sixteenth and the twentieth days (*Ogorodniy, 1939; Sandreuter, 1951*). *Ascarelli (1895)* attributed a consistent decrease on the nineteenth day to stagnation



of the blood cells in the allantoic vessels when pulmonary respiration begins. A temporary decline in the red cell count of the duck (*Anas platyrhynchos*) embryo has been observed on both the twentieth and the twenty-fourth day of incubation (*Fetischeva, 1939*).

Sümege (1932) concluded that the chick embryo's red blood count on the nineteenth incubation day varies with body weight. According to Fennell (1947), resistance to lymphomatosis is correlated with a high embryonic erythrocyte count.

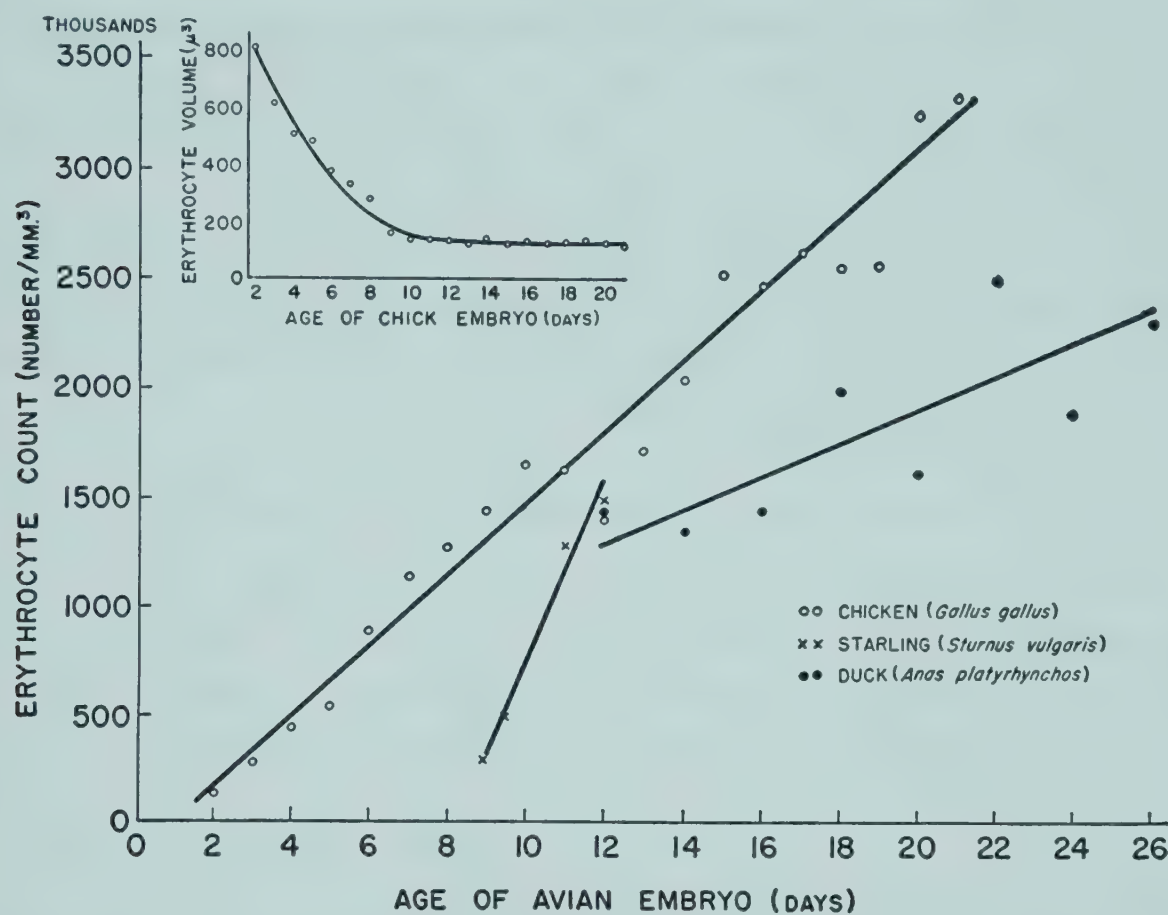


Fig. 229. Erythrocyte count in the blood of avian embryos at successive stages of incubation.

A, starling (*Sturnus vulgaris*), after data of Sandreuter, 1951; B, chicken (*Gallus gallus*), averaged after data of Ascarelli, 1895; Sümege, 1932; Ogorodniy, 1939; Rychter, Kopecky, and Lemez, 1955; C, duck (*Anas platyrhynchos*), after data of Fetischeva, 1939.

*Insert:* changes in the erythrocyte volume of the blood of the developing chick embryo. (After the data of Rychter, Kopecky, and Lemez, 1955.)

By the hatching date, the erythrocyte count of the chick has increased to at least 2,500,000 or 3,000,000, or to more than 90 per cent of the adult value (*Ascarelli, 1895; Kelly and Dearstyne, 1935; Blount, 1939; Romanoff, 1948; Sandreuter, 1951*). The male chicken's blood contains a higher average number of erythrocytes than the female's, both at hatching time (*Romanoff, 1948*) and in adult life (*Sandreuter, 1951*).

After hatching, the red blood count of the chicken approximates the adult value on the tenth day of postembryonic life (*Blount, 1947, pp. 160–163*) and remains constant until the twentieth day. It then decreases some-



what and does not return to the adult level until the bird is about 3 months old (*Sandreuter, 1951*).

The pattern of erythrocyte increase in the starling (*Sturnus vulgaris*) is considerably different. From about 300,000 on the ninth incubation day, the red blood cell count rises to about 1,400,000 at hatching, or to less than 60 per cent of the adult value. The definitive level is attained before the twentieth post-hatching day, is surpassed for a period of time extending from the twenty-second to the thirty-sixth day, and again prevails by the fiftieth day (*Sandreuter, 1951*). The period of maximum erythrocyte count coincides with the time when the bird is learning to fly. An elevated red blood count has also been found to occur in four races of pigeons from the fourteenth to the seventy-fifth post-hatching days (*Riddle and Cauthen, 1938*), when the power of flight is developing.

Incubation of the chick embryo under increased atmospheric pressure (40 pounds) retards the formation of erythrocytes, as evinced by a sub-normal red blood cell count (less than half the normal value) on the tenth day (*Flemister and Cunningham, 1940*).

*The Hemoglobin of Embryonic Red Blood Cells*

The actual amount of hemoglobin present in the embryo increases throughout the incubation period. The average total quantities found in chick and duck (*Anas platyrhynchos*) embryos on successive days are recorded in the accompanying table. According to *Ascarelli (1895)*, the

Incubation Age (days)	Hemoglobin per Embryo (mg.)	
	Chicken ( <i>Gallus gallus</i> )	Duck ( <i>Anas platyrhynchos</i> )
	( <i>Schönheyder, 1938; Sendju, 1927; Ogorodniy, 1939</i> )	( <i>Fetischeva, 1939</i> )
1.5	Trace	
3	0.2	
4	0.8	
5	2.0	
6	4.3	
7	7.6	
8	15.7	
9	18.8	
10	21.9	
12	23.0	34.0
14	26.9	37.5
16	36.0	30.0
17	32.0	
18	38.0	43.0
19	34.0	
20		35.0
22		48.0
24		52.0
26		42.0



amount of hemoglobin present in the chick embryo increases from about 20 milligrams on the seventh day to 52 milligrams on the twentieth day. Ramsay (1950) found that the hemoglobin in the entire incubated chicken egg rose from 26 milligrams to 208 milligrams between the eighth and twenty-first days. The hemoglobin content of the 2-day-old hatched chick is said to be about 170 milligrams (Schönheyder, 1938).

The concentration (percentage) of hemoglobin in the chick embryo's blood does not change greatly and is not far below the normal adult level from the twelfth day to hatching, when the average hemoglobin reading of 63.5 per cent indicates that about 90 per cent of the definitive concentra-

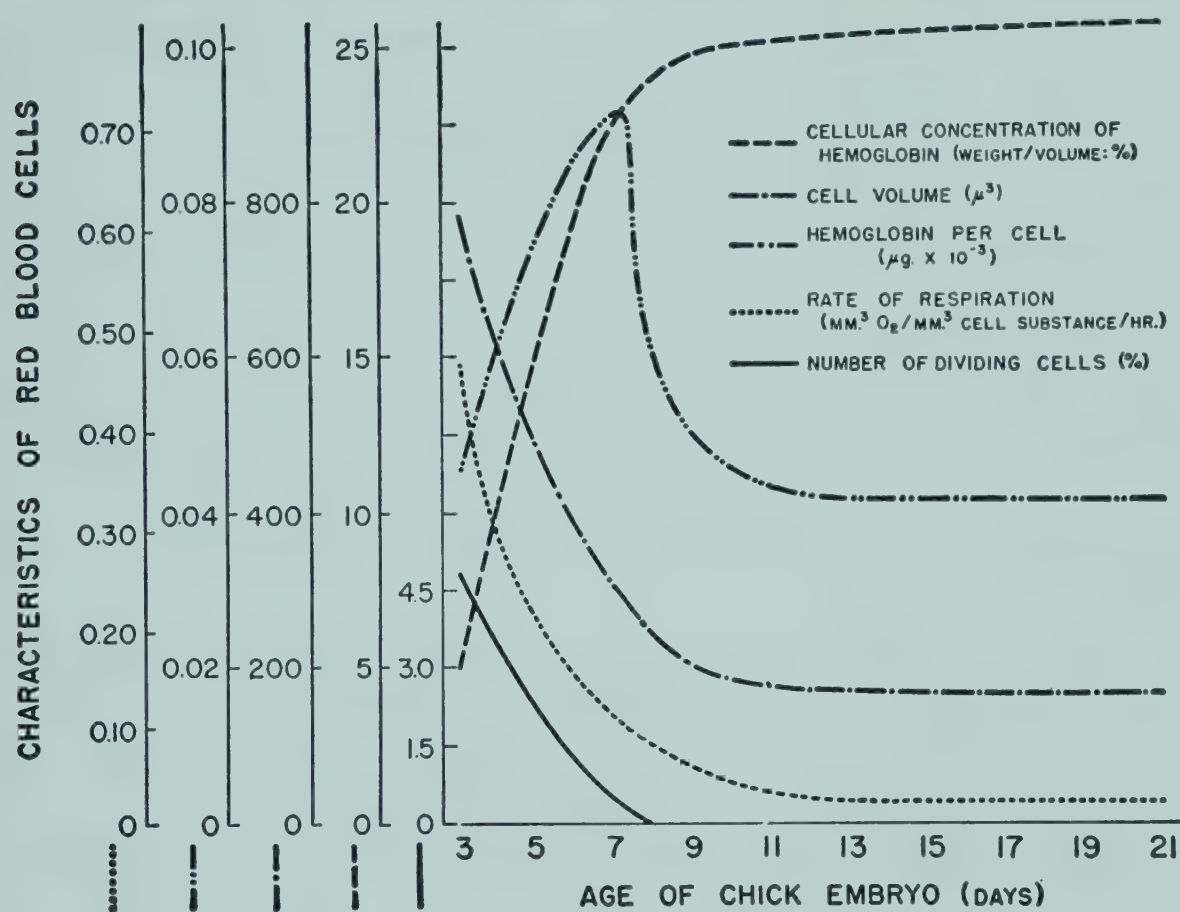


Fig. 230. Progressive changes in various attributes of the chick embryo's red blood cells during incubation. The curves represent the amount of hemoglobin per cell, the cell volume, the cellular concentration of hemoglobin (weight/volume: per cent), the percentage of dividing cells, and the rate of respiration. (Redrawn with modifications after O'Connor, 1952b.)

tion is present (Sandreuter, 1951). Incubation at an atmospheric pressure of 40 pounds has been found to inhibit the formation of hemoglobin to such an extent that less than half the normal concentration is present on the tenth day (Flemister and Cunningham, 1940).

In the starling (*Sturnus vulgaris*), the hemoglobin reading is only 33 per cent at hatching. During the first 3 weeks of postembryonic life, there is a rapid increase to the average adult level of about 100 per cent (Sandreuter, 1951).

During incubation, there are also changes in the concentration of hemoglobin within the chick's red blood cells. As Fig. 230 shows, there is a



rapid increase in the amount of hemoglobin per cell from the third to the seventh day of incubation and an equally rapid decrease from the seventh to the tenth day, after which the value remains constant. The fall in cellular concentration coincides with the appearance of the definitive strain of cells in the circulation. The smaller size of these cells probably accounts—in part at least—for the simultaneous drop in cell volume (cf. Fig. 230), and this reduction in cell volume in turn explains the fact that the concentration of hemoglobin, expressed as a weight per volume percentage (cf. Fig. 230), has been found not to decrease after the seventh day (O'Connor, 1952b). The definitive erythrocytes are small and poor in hemoglobin from the fifth incubation day on according to Rychter (1955).

Despite their relatively low hemoglobin content, as compared with the primitive red cells, the definitive cells are hyperchromatic for the duration of embryonic life; the amount of hemoglobin increases at a more rapid rate than the number of erythrocytes (Sandreuter, 1951). The color index of the chick's erythrocytes during the last half of incubation averages about 1.6 (Sümegei, 1932; Ogorodniy, 1939); it may reach a maximum of 1.8 (Ogorodniy, 1939). Hyperchromatic cells are found for at least a week after hatching (Sandreuter, 1951). In the starling (*Sturnus vulgaris*), the highest color index is seen on the first 2 days after hatching, and the hyperchromaticity of the erythrocytes persists until the bird is a month old (Sandreuter, 1951).

Rychter, Kopecky, and Lemez (1955) made a study of the circulating blood volume in chick embryos from the second incubation day, approximately 8 hours after blood circulation begins, until hatching. The ratio between plasma, blood volume, and amount of hemoglobin and a unit of weight of embryo plus membranes shows the relative amount of circulating plasma and blood decreases during development. This contrasts with the fairly constant relative hemoglobin from the eighth day of incubation to hatching. The fall in relative amount of plasma is 55 per cent, of blood 50 per cent, and of hemoglobin 15 per cent during the last two thirds of incubation (Fig. 231).

It is probable that the hemoglobin formed during the chick's incubation period, and especially during the early stages of development, differs from the hemoglobin of the adult bird. Embryonic hemoglobin is said to be less stable (Hugounenq and Morel, 1905) and to contain more iron (Ogorodniy, 1939). Its oxygen dissociation curve shows that it has a higher affinity for oxygen (Hall, 1934), as if in compensation for the conditions of low oxygen tension prevailing within the incubating egg. The oxygen affinity of the chick's hemoglobin decreases progressively from the tenth incubation day to the fortieth postembryonic day, when the adult value is approximated. It appears, therefore, that the embryonic form of the substance is gradually replaced by the definitive form (Hall, 1934). It has been sug-



gested that the former is produced by the yolk sac and the latter by the spleen and bone marrow (*Dawson, 1936*).

Iisu, Stern, and McGinnis (*1952*), experimenting on vitamin B<sub>12</sub> deficient blood of 18- to 21-day chick embryos, found that the hemoglobin and red cell counts were raised significantly by injecting vitamin B<sub>12</sub> into the egg.

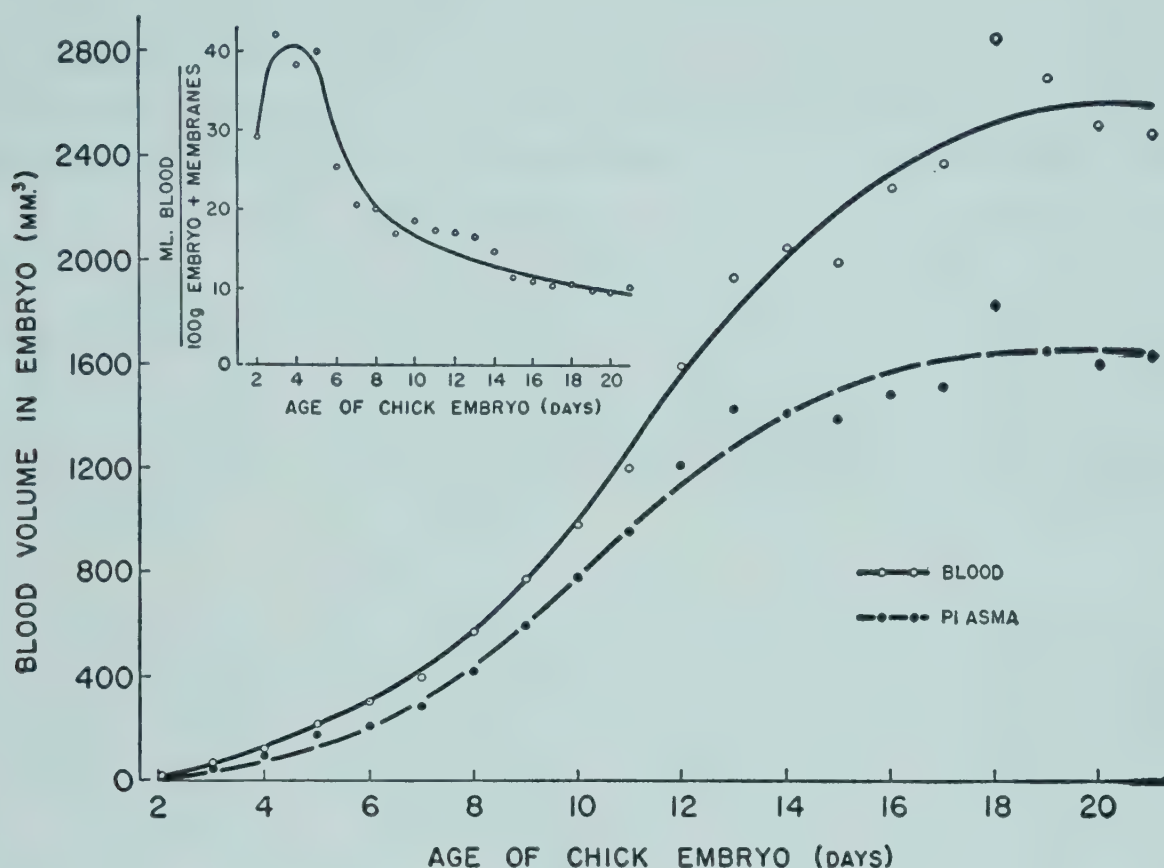


Fig. 231. Volume of circulating blood and plasma in the chick embryo at successive stages of development. (Compiled from the data of Rychter, Kopecky, and Lemez, 1955.)

Insert: blood volume in relation to weight of the embryo and its membranes.

### *The Respiration of Embryonic Red Blood Cells*

In the chick embryo, oxygen uptake by the red blood cells diminishes during incubation. There is a particularly rapid decrease during the first week of incubation (*O'Connor, 1952a*), as may be seen in Fig. 230. The high initial consumption of oxygen has been related to the mitotic division of cells (cf. Fig. 230), which in turn has been related to the catabolism of carbohydrate (*O'Connor, 1951, 1952a, 1952b*).

The respiration of the chick's red cells continues to decrease during the last 2 weeks of incubation. Leibsohn (*1940*) found that the oxygen consumption of a given volume of erythrocytes at the end of the development is only about 30 per cent as high as on the seventh day of incubation (Fig. 232). A particularly sharp decrease occurs during the last week of embryonic life. Boyer (*1950*), using whole blood, observed that the respiratory rate exhibits a similarly rapid fall from the fourteenth to the nineteenth day, after which time it remains constant, at least for the first week



of postembryonic life (cf. Fig. 232). According to Boyer, the drop in oxygen consumption during development may be explained in part by the progressive accumulation of hemoglobin having a lower affinity for oxygen than the early hemoglobin. He suggested, however, that it may perhaps be explained in equally large part by the increasing number of mature or maturing red blood cells in relation to the number of young cells with a higher metabolic rate. Embryonic tissues other than blood exhibit much the same downward trend in respiratory activity with age (*Romanoff, 1943e*), and it is possible that the decrease in intensity of erythrocyte respiration reflects not only the maturation of erythrocytes, but also the maturation of the organism as a whole (*Leibsohn, 1940*).

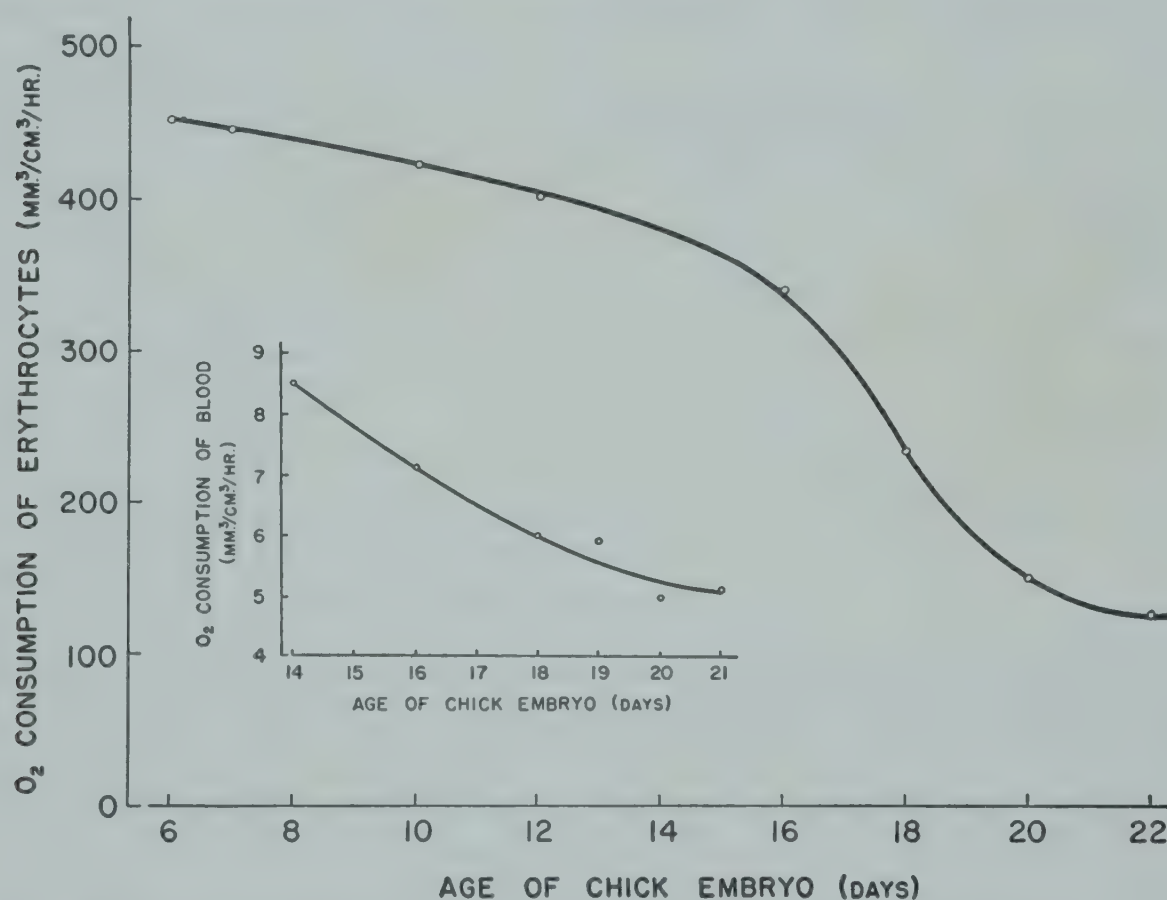


Fig. 232. Curves showing the changes in the oxygen consumption of the chick embryo's erythrocytes and whole blood during successive stages of development. (Redrawn with modifications after Leibsohn, 1940; Boyer, 1950.)

### The Embryonic White Blood Cells

The white blood cells of avian embryos have been less extensively studied than the red blood cells. This is not surprising in view of their minor role in the total embryonic blood picture. Information is available, however, regarding the changing numbers and proportions of the various types of leucocytes during the course of incubation.

#### *The Maturation of the Leucocytes*

Like the red blood cells, the leucocytes of all types pass through a series of developmental stages before attaining their mature forms. These stages, which have been described in great detail in the literature, are



characterized by changes that affect, mainly, the staining properties of the cell, the character of the cytoplasmic organelles, and the morphology of the nucleus.

A description of all the immature white blood cells of the avian embryo cannot be given here for want of space. It may be briefly noted, however, that the characteristic granulation of the granulocytes distinguishes these cells at an early stage. A convenient classification for immature granulocytes is that adopted by Sandreuter (1951), who recognized promyelocytes I, promyelocytes II, myelocytes, and metamyelocytes in the eosinophilic, pseudoeosinophilic, and basophilic series. In the eosinophilic series, the

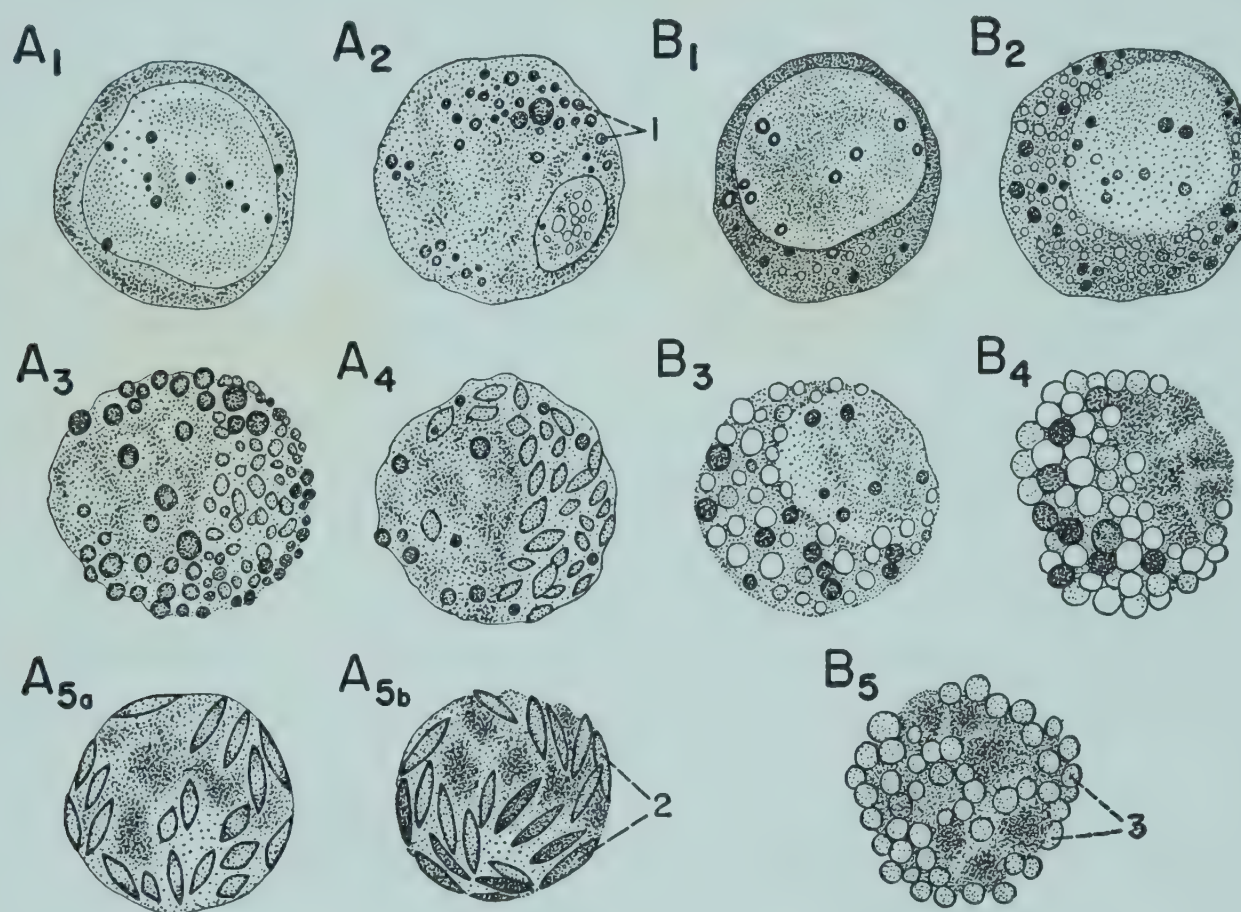


Fig. 233. Schematic representation of successive stages in the development of the characteristic granules of A, the eosinophilic and B, the pseudoeosinophilic leucocytes of the chick embryo. (Redrawn with modifications after Sandreuter, 1951.)

A<sub>1</sub> and B<sub>1</sub>, promyelocytes I; A<sub>2</sub> and B<sub>2</sub>, promyelocytes II; A<sub>3</sub>, A<sub>4</sub>, B<sub>3</sub>, B<sub>4</sub>, megalocytes; A<sub>5a</sub>, A<sub>5b</sub>, B<sub>5</sub>, metamyelocytes. All  $\times 1600$ .

promyelocytes I contain only amorphous "progranules" of different staining properties from the definitive, fusiform granules; the latter first appear in the promyelocytes II and begin to assume their final shape in the myelocyte stage (Fig. 233). The amorphous granules of the pseudoeosinophilic cells are recognizable from the beginning in the pseudoeosinophilic promyelocytes I, although most of the granules are achromatic at this stage (Sandreuter, 1951). Their staining reaction is evident in the promyelocytes II. As the granulocytic cells increase in maturity, their nuclei change in shape, becoming successively round, semispherical, kidney-shaped, and horse-shoe-shaped (Sugiyama, 1926; Sandreuter, 1951).



The lymphocytes and monocytes undergo less pronounced maturation changes, since they are derived from cells that are not unlike them. There are some differences in staining properties, size, and the proportional amount of cytoplasm. Sandreuter (1951) distinguished prolymphocytes and promonocytes as the forms immediately preceding the definitive cells.

*The White Blood Cell Count*

Leucocytes are not found in any great number in the circulating blood of the chick embryo until the last days of incubation (Sugiyama, 1926; Dawson, 1936; Fennell, 1947). The white blood cell count (granulocytes) ranges between 100 and 150 per cubic millimeter on the fourteenth to sixteenth days (Weller and Schechtman, 1949). Just prior to hatching, the number of leucocytes of all kinds may vary from 2,000 to 9,000 per cubic millimeter (Blount, 1939; 1947, pp. 160-163), and it is rarely over 10,000 per cubic millimeter on the day after hatching (Kelly and Dearstyne, 1935; Blount, 1939). During the next 10 days, the number of leucocytes rapidly increases to a maximum of 20,000 or 30,000 per cubic millimeter (Blount, 1947).

The eosinophilic cells are the first leucocytes to enter the blood stream, reportedly migrating into the chick embryo's blood vessels as early as the fifth to seventh day of incubation (Sugiyama, 1926). They are the only white cells present until the end of the second week of incubation. Some of the other types are formed in the hematopoietic foci several days before they enter the circulation, as the accompanying data indicate (Sandreuter,

Cell Type	Time of Appearance (incubation day)				
	Embryonic Mesenchyme	Liver	Spleen	Bone Marrow	Blood
Pseudoeosinophile	18-21	14-18	12	14	18
Basophile	9	14-18	12	13-14	14
Lymphocyte	11-12	18	12-18	18	14
Monocyte		18	14-18	18	18

1951). In the starling (*Sturnus vulgaris*), there are no leucocytes other than eosinophiles in the blood stream until the hatching day (twelfth to thirteenth incubation day), when pseudoeosinophiles and basophiles (present in the spleen from the eighth incubation day) are seen in small numbers in the circulating blood as well as in the liver and bone marrow. Lymphocytic cells also make their first appearance at this time in the blood, liver, and spleen. Monocytic cells are not present until 5 or 6 days after hatching, or 18 days from the beginning of incubation; plasma cells appear in the starling (*Sturnus vulgaris*), as in the chick, 41 days after incubation starts (Sandreuter, 1951). In the two species, therefore, the various leucocytes



TABLE 10

Changes in the Differential White Blood Cell Count in the Chick (*Gallus gallus*) and Starling (*Sturnus vulgaris*) during Embryonic Period \*

Type of White Blood Cells	Age of the Chick Embryos				Age of the Starling Embryos			
	10 Days	12 Days	14 Days	18 Days	Hatched	8 Days	10 Days	12 Days
	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)	(per cent)
Basophiles			1.0	5.0	6.5			3.0
Pseudoeosinophilic myelocytes								3.0
Pseudoeosinophiles			1.0	9.5	9.0			5.0
Eosinophilic myelocytes						2.0	3.0	27.0
Eosinophiles	33.0	57.5	43.5	55.3	52.5	16.0	45.0	47.0
Prolymphocytes			25.0	10.5	24.0			10.0
Lymphocytes								3.0
Promonocytes				4.0	5.0			
Monocytes					3.0			
Hemocytoblasts	67.0	42.5	29.5	13.5		82.0	52.0	12.0
								2.0

\* Chiefly after Sandreuter (1951).



enter the circulation on approximately corresponding days from the onset of development.

This indicates that the differential white blood count of the avian embryo is unlike that of the adult bird. The embryonic white blood picture is characterized by an eosinophilic leucocytosis; this condition prevails until the fifth post-hatching day in the chick and until the tenth day in the starling (*Sturnus vulgaris*) and is much more pronounced in the latter species (Sandreuter, 1951). A rapid increase in lymphocyte production starts at the time of hatching and brings the proportion of these cells to or above the adult level in both birds within the first month of postembryonic life. Pseudoeosinophiles, basophiles, monocytes, and plasma cells are present in approximately definitive proportions from the time of their appearance. A few young and immature white blood cells are found prior to and shortly after hatching, but in larger numbers in the starling than in the chick (Sandreuter, 1951). Table 10 summarizes the changes in the differential white blood count of the chick and the starling during the latter part of incubation and the early postembryonic period, as determined by Sandreuter (1951).

In the 13- to 15-day chick embryo, as in the adult fowl, a single injection of adrenal cortical extract produces a marked granulocytosis within a few hours after administration. Although this substance also causes a transient lymphopenia in the adult, it appears to have no effect on the numbers of embryonic lymphocytes or hemocytoblasts (Weller and Schechtman, 1949).

### INTRAEMBRYONIC BLOOD VESSELS

The formation of intraembryonic blood vessels begins only a short time after extraembryonic vascularization starts and proceeds rapidly. In fact, a sufficient number of primitive vessels are present at the stage of 16 or 17 somites to permit the blood to make a complete circuit through the embryonic body and the area vasculosa (Sabin, 1920).

Despite the fact that vascularization progresses centripetally in the blastoderm as a whole, the endothelial tissue forming the walls of the intraembryonic vessels does not grow in from the extraembryonic area, as was once believed; on the contrary, it differentiates *in situ* (Rückert and Mollier, 1906; McClure, 1921). The theory of the invasion of the pre-vascular tissue from the area opaca, proposed by His (1868, *p.* 99; 1882, 1900), was supported by Türistig (1884), Vialleton (1892*b*), Gräper (1907), and others. It has been successfully demonstrated, however, that experimental separation of the embryonic area from the area opaca at an early stage does not prevent the formation of intraembryonic blood vessels (Hahn, 1908, 1909; Miller and McWhorter, 1914; Reagan, 1915, 1917).



The centripetal development of blood and vessels is due to the fact that the mesoblastic cells which have migrated farthest from the primitive streak are the most mature and naturally differentiate first (*Grodzinski, 1934a*).

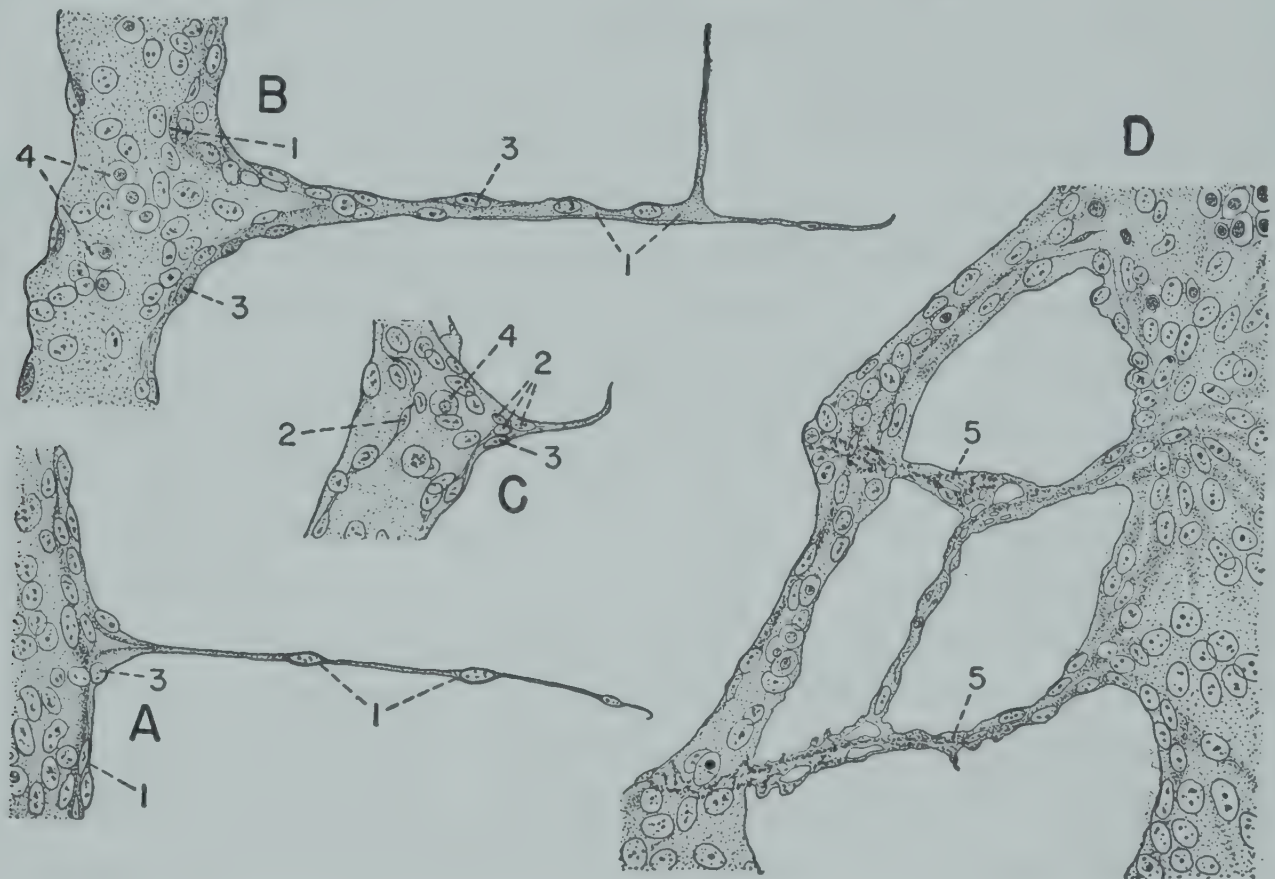
The primitive vascular system of the embryonic body—a system including, among other vessels, the dorsal aortae, the aortic arches, the cardinal veins, and the pulmonary arteries and veins, as well as the endothelial lining of the heart—is formed, therefore, in much the same manner as the extraembryonic vascular system. Solid aggregates of cells differentiate from mesenchyme along the future course of a vessel and become luminate, either before or after anastomosing with one another (*Jolly, 1939–40*).

It is not known exactly how long, during incubation, mesenchyme continues to give rise to these cellular aggregates of prevascular tissue, which may be designated angioblasts (rather than hemangioblasts, since their blood-forming capacities are doubtful or at least limited). *Sabin (1917b, 1922)* was inclined to the view that the differentiation of new angioblasts from mesenchyme occurs for only a short time, after which all new blood vessels arise from the growth or proliferation of older angioblasts or endothelium. She distinguished three periods in intraembryonic vascularization: a stage prior to circulation, when the primitive vessels form from angioblasts; an intermediate stage, during which the division and growth of already formed vessels and the progressive differentiation of new vessels accomplishes the extension of the vascular system throughout the body; and a final stage during which new vessels are formed only from pre-existing endothelium (*Sabin, 1922*). Opposed to this view is the opinion that new angioblasts may continue to differentiate almost indefinitely, or at least until relatively late in ontogeny (*Reagan, 1917*).

The growth and branching of existing vessels are brought about largely by the process of “sprouting” (*Sabin, 1922*)—that is, by the proliferation of solid strands of endothelial cells in which there form lumina continuous with the lumen of the parent vessel. Some of the stages in the sprouting of a capillary are shown in Fig. 234-A, B, and C. It has been suggested that already formed vessels may also grow by the addition of mesenchyme cells or of angioblastic and endothelial cells that have differentiated locally from mesenchyme (*Reagan, 1917*). Sinusoids, the venous spaces which are seen during the early development of the chick embryo's liver, mesonephros, heart, and suprarenal capsules, are formed by the growth of endothelial cells and their close application to, or intercrecence with, parenchymal tissue (*Minot, 1900b*). Sinusoids are much wider and more irregular in diameter than capillaries, and their endothelial nuclei are fewer and farther apart (Fig. 235). Although formed simultaneously with capillaries, sinusoids are eventually replaced by capillaries and seem to arise in response to particular though transitory physiological conditions.



In general, definitive blood vessels, with the exception of those formed during the earliest or primitive period, are probably preceded by capillary plexuses, out of which the vessels are established under the influence of the circulation (Hughes, 1934, 1935). It is not unusual for a definitive vessel to replace a primitive vessel, which disappears; or for a definitive vessel to appear only after several vessels have formed and degenerated in



**Fig. 234.** Development of veins from pre-existing vessels. Venous sprouts developing (A, B, C) directly from pre-existing vessels of various sizes in the area pellucida of the same 4-day chick embryo, and degenerating (D) after replacement by more permanent vessels. (Adapted from Sabin, 1922.)

A, endothelial sprout from the wall of the median anterior vein; only a portion of the wall of that vessel is shown; B, branched endothelial sprout from a smaller vein; C, small sprout with nuclei aggregated at its base; D, degeneration of venous sprouts from large, normal vein (at right), showing preliminary collapsing of the endothelium of the sprout and the degeneration and death of both adventitial and endothelial cells. Note that whereas the endothelial sprouts from the veins possess a continuous protoplasm, the degenerating vessels break down with their protoplasm becoming discontinuous, perforated, and tenuous. All  $\times 250$ .

1, endothelium; 2, endothelial nucleus; 3, adventitial nucleus; 4, red blood cell; 5, degenerating endothelium.

succession. The degeneration of endothelial tissue, therefore, is an important aspect of vascularization. Sabin (1922) has described the normal destruction of vessels as beginning with a collapse of the walls that obliterates the lumen, after which the endothelium and the adventitia degenerate (cf. Fig. 234-D). Because of the changing relationships between embryonic vessels, the direction of blood flow may be profoundly altered or even reversed during the course of development.



The course taken by a blood vessel is probably determined to some extent by the presence of pre-existing structures, around which or along which capillary plexuses may develop. The condensation of a capillary plexus around a nonvascular organ appears to result from a type of contact response (Hughes, 1934), similar to that exhibited by nerves (see Chapter 4).

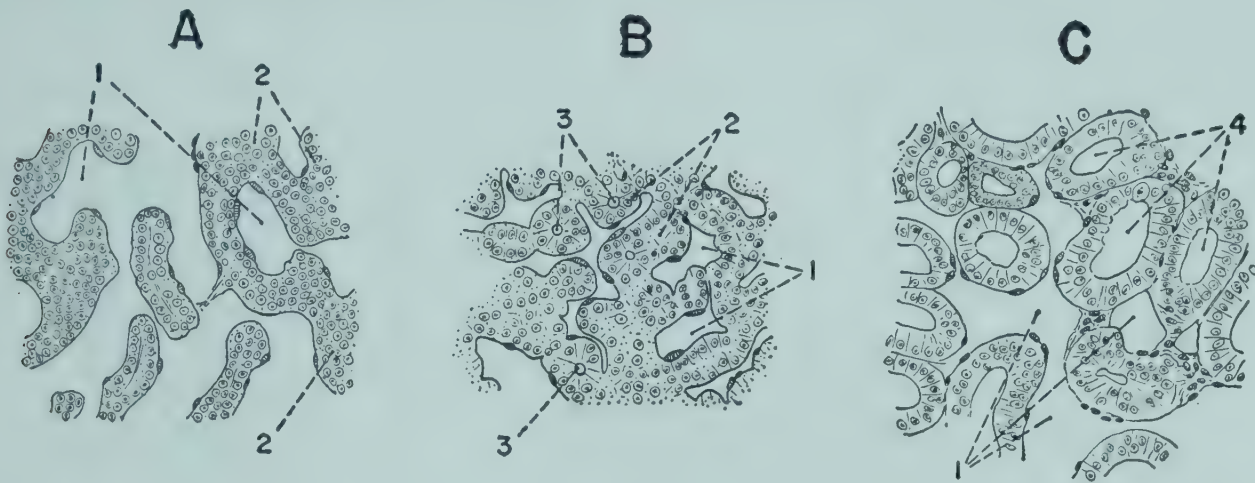


Fig. 235. Sinusoids in the liver and mesonephros of the developing chick embryo. (Redrawn with modifications after Minot, 1900b.)

A, large sinusoids in the liver of the 6-day chick embryo; B, sinusoids of diminished size in relation to the numerous hepatic cylinders in the 11-day chick embryo; C, sinusoids in the mesonephros of the 11-day chick embryo. All  $\times 105$ .

1, sinusoid; 2, hepatic cylinder; 3, bile duct; 4, mesonephros or Wolffian tubules.

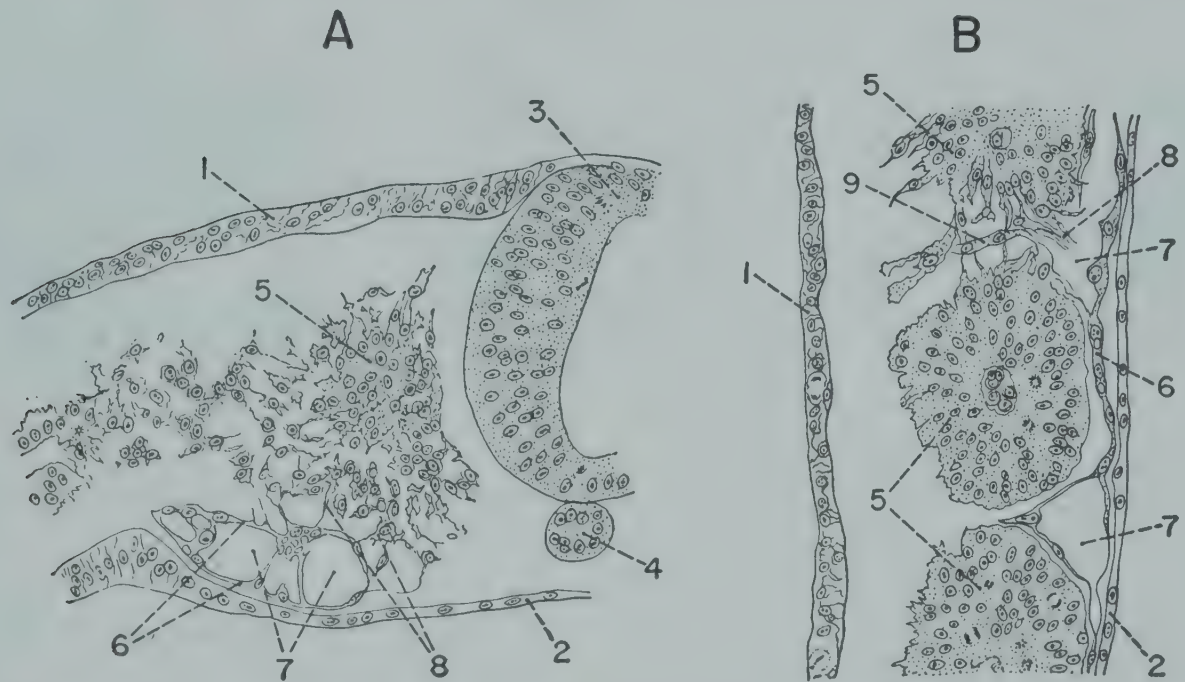
### The Aorta

The aorta, the principal arterial vessel of the body axis, is the first intraembryonic blood vessel to make its appearance. It arises from paired primordia, the dorsal aortae, which fuse throughout all but a small anterior portion of their extent to form the definitive, single aorta. Short ventral aortae also form during embryogeny as cephalic prolongations of the heart; these become connected with the dorsal aortae by the six pairs of aortic arches. The aortic arches are not all present simultaneously, because they appear in succession and because some of them are only transitory.

The aortae arise from angioblasts that develop *in situ* (Hahn, 1909; Reagan, 1917; Jolly, 1939–40), and not from outgrowth of endocardial tissue. As previously noted, aortal anlagen are present in the chick embryo of five somites. Both ventral and dorsal aortae are clearly visible at the 6-somite stage. Chains of angioblasts representing the ventral aortae continue forward only a short distance from the paired cardiac primordia, which are beginning to come into contact in the midline. The dorsal aortae can be followed from the level of the heart posteriorly along the ventrolateral border of the somites; these vessels are only partially luminate, and the plexus of solid angioblasts of which they largely consist is continuous with the plexus of angioblasts in the area pellucida (Sabin, 1917b).



The plexus of angioblasts representing the aortae increases by cell division and by the differentiation of new cells (*Sabin, 1917b*). In chick embryos of eight to twelve somites, the endothelial cells of the discontinuous dorsal aortae have been observed to differentiate and detach themselves from the mesoderm closest to the axial line (*Jolly, 1939-40*). Cephalic to the first somite, the cells arise, of course, from unsegmented mesoderm. At the level of the somites, they delaminate from the ventral surfaces of the



**Fig. 236.** The early development of the aorta in the chick embryo. (Redrawn with modifications after *Jolly, 1939-40*.)

A, transverse section through the second somite of a 12-somite embryo; B, paramedian sagittal section of a 10-somite embryo at the level of the third to fifth somites. Both  $\times 200$ .

1, ectoderm; 2, endoderm; 3, neural tube; 4, chorda; 5, somite; 6, primordium of wall of aorta; 7, lumen forming in aorta; 8, anastomosis between aorta and somite; 9, primordium of intersomitic artery.

myotomes and from the internal portion of the lateral plate mesoderm. Even after lumina have started to form in the aortae, the aortic endothelium may remain connected with the somites by anastomoses (*Fig. 236-A*). Similar connections have been seen (*Jolly, 1939-40*) between the somites and the intersomitic arteries (*Fig. 236-B*), which are branches of the dorsal aortae extending dorsally between the somites.

Anteriorly, both dorsal and ventral aortae extend themselves into the head region. Early in the second day (*Baer, 1827*), at about the 10-somite stage (*Duval, 1889; Sabin, 1917b*), the dorsal aortae become continuous with the ventral aortae just posterior to the optic vesicles (*Fig. 237*). The connecting segments curve dorsoventrally around the anterior end of the fore-gut and are regarded as the first, most anterior pair of aortic arches. It is at this stage that the heart begins to beat (*Sabin, 1917b*), although blood does not yet circulate.

In general, the formation of lumina in the solid angioblastic cords of



the aortae progress in the cephalocaudal direction (*His*, 1868, *p.* 100; *Vialleton*, 1892*b*; *Jolly*, 1939–40). The process, however, is extremely irregular, and the aortae may be well formed at levels caudal to points where they are nonluminate or represented only by a few angioblasts (*Jolly*, 1939–40). The lumen probably appears first, or is largest, where the intersegmental arteries are given off (cf. Fig. 236-B), and it may be more or less reticulated in the beginning (cf. Fig. 236-A).

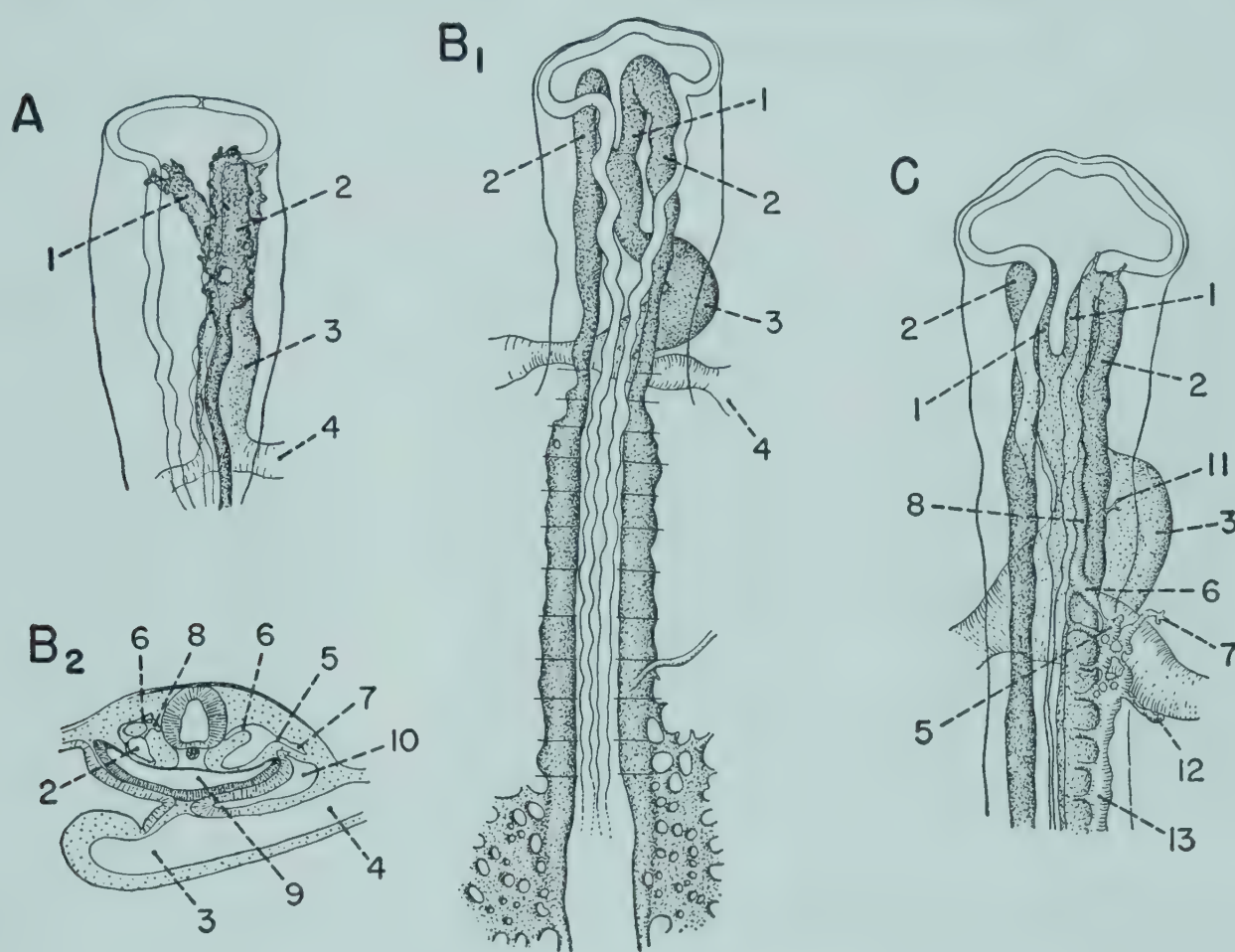


Fig. 237. Early development of the aortae in the chick embryo. (Redrawn with modifications after Sabin, 1917*b*.)

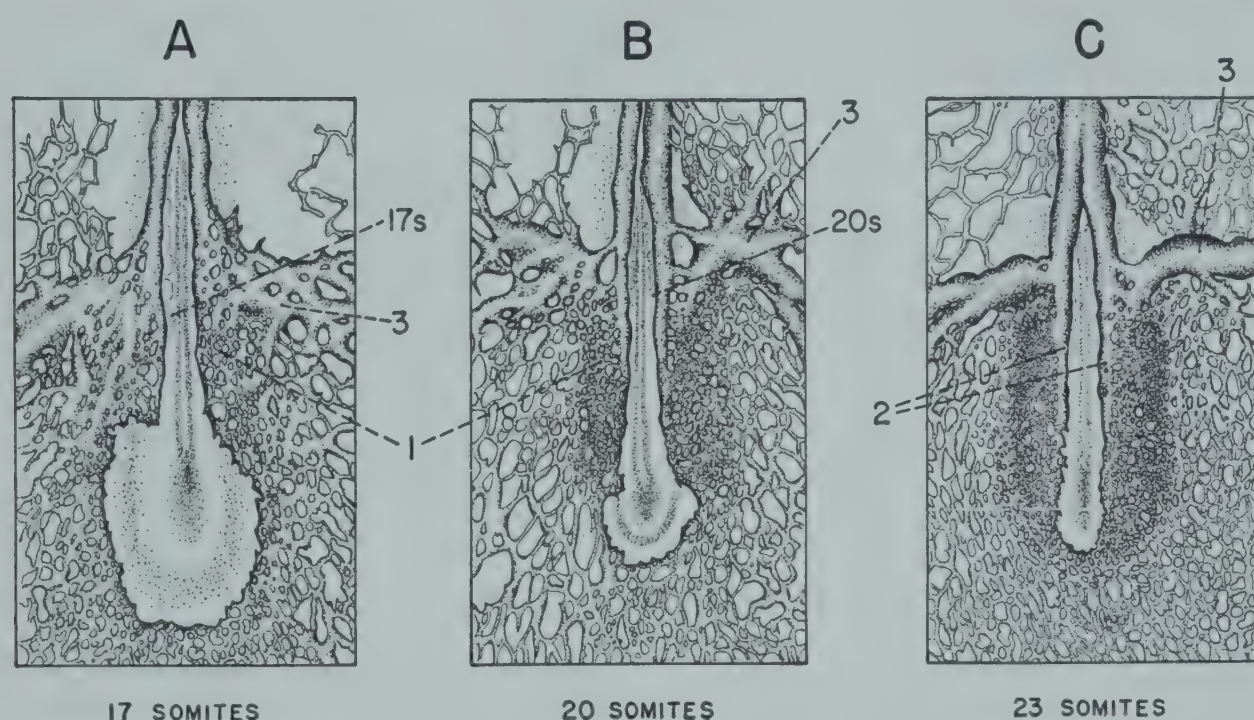
A, dorsal aorta, shown on one side only, 9-somite stage ( $\times 35$ );  $B_1$ , the same, 12-somite stage ( $\times 35$ );  $B_2$ , the same, as seen in transverse section through the first interspace ( $\times 40$ ); C, dorsal aortae, 14-somite stage ( $\times 35$ ).

1, ventral cephalic aorta; 2, dorsal cephalic aorta; 3, heart; 4, omphalomesenteric vein; 5, anterior cardinal vein; 6, transverse vein of the first interspace; 7, vein of the somatopleure; 8, primitive vessel of the rhombencephalon (*vena capitis medialis*); 9, pharynx; 10, coelom; 11, artery of the somatopleure; 12, duct of Cuvier; 13, posterior caudal vein.

In the 10-somite chick embryo, the dorsal aortae extend to the posterior limit of the segmented region. At this stage, as *Vialleton* (1892*b*) noted, the aortae are simple tubes down to the level of the fourth somite, where they begin to have capilliform connections with the plexus of the area pellucida. Caudal to the seventh somite, the aortae merge freely with this plexus, which encircles the terminal portion of the embryo rather widely, without encroaching upon the median region. As somites are added, the aortae are always in free communication with the extraembryonic plexus at



the level of the last five or six somites (*Evans, 1909b*). Gradually, the terminal portions of the aortae extend caudad, and they reach well below the last somite by the 17-somite stage (Fig. 238-A). The omphalomesenteric arteries, resolving from the extraembryonic capillary plexus (*Mansawa, 1927*), are connected with the aortae immediately above the plexiform portions of the latter. Blood now begins to enter the heart through the omphalomesenteric veins and to be returned to the area vasculosa through the aortae and the omphalomesenteric arteries. By the 20-somite stage (Fig. 238-B), the aortae extend to the level of the end bud, and the region where they send out lateral capillaries lies almost entirely below the last



**Fig. 238.** Differentiation of the caudal portions of the dorsal aortae in the developing chick embryo. (Redrawn with modifications after *Evans, 1909b*.)

A, capillary plexus opposite the last somite in the 17-somite embryo, ventral view; B, trunks of the lower aortae forming at the inner margin of the extended capillary plexus, ventral view, 20-somite stage; C, further differentiation of the lower aortae and caudal extension of the capillary plexus, ventral view, 23-somite stage. All  $\times 25$ .

1, capillary plexus; 2, lower aortae; 3, omphalomesenteric artery.

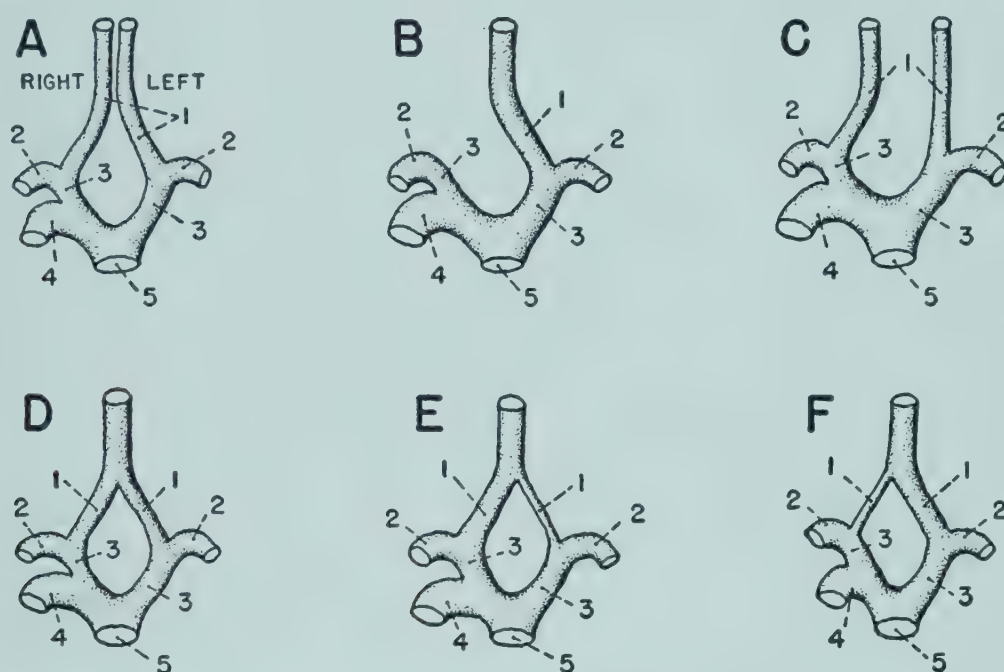
somite. When the embryo has twenty-two (*Vialleton, 1891*) to twenty-four (*Evans, 1909b*) somites, the aortae reach the posterior tip of the body (Fig. 238-C). According to *Evans (1909b)*, a few persisting capillaries connect the aortae with the posterior vitelline vein.

The dorsal aortae are brought into close apposition as the coelom widens and the splanchnopleure approaches the midline in the course of forming the dorsal mesentery. The aortae begin to fuse together soon, approximately after their terminal portions differentiate. Their fusion starts at the approximate level of the vitelline veins and proceeds caudad. The plates of *Duval (1889)* show that a single aorta is present opposite the fifth to tenth somites, inclusive, of the 27-somite chick and can be traced to a point below the twenty-first somite at the 40-somite stage. At the end of the fourth day, the dorsal aortae have fused together from the level of the



heart to that of the posterior limb buds. Their fusion in the caudal region is completed during the fifth day.

In the trunk region of the embryo, the dorsal aorta develops several sets of branches (*Sabin, 1917b*). First, there are the dorsal or dorsolateral intersomitic branches; these give rise to the cardinal veins and also to dorsal or medial branches (segmental spinal arteries) to the spinal cord. In addition, there are formed a few secondary dorsolateral branches to the cardinal veins; lateral arteries to the limb buds; and lateral arteries to the kidneys.



**Fig. 239.** Species differences in the carotid arteries of adult birds. (Redrawn with modifications after Garrod, 1873.)

Normal arrangements of the carotid arteries of birds include two parallel carotid arteries of equal size, *A*, found in the majority of avian species: a single (left) carotid artery; *B*, found in all passerine birds: two nonparallel carotid arteries; *C*, found in some parrots: a single carotid artery formed from the union of the right and left carotid arteries; *D*, as in the European bittern (*Botaurus stellaris*): two carotid arteries of unequal size, with either the left, *E*, or the right, *F*, smaller, as in the sulphur-crested cockatoo (*Cacatua sulphurea*) and flamingos (*Phoenicopteridae*), respectively.

1, carotid artery; 2, subclavian artery; 3, innominate artery; 4, arch of the aorta (IV); 5, aortic trunk.

The history of the anterior paired dorsal aortae is intimately interrelated with that of the aortic arches. The portions of the aortae connecting the dorsal ends of the first three pairs of aortic arches persist as the dorsal carotid arteries, although the first two pairs of aortic arches disappear. When the heart and the aortic arches begin to migrate backward, the aortae behind the third aortic arches become longer and narrower. On the left side, the entire segment of aorta between the third and sixth arches atrophies, along with the left fourth arch; on the right side, however, the aorta degenerates only between the third and fourth aortic arches, both of which remain permanently. Degeneration begins in the outer layers of the aortic wall, after the lumen is obliterated. Meantime, the single, median dorsal



aorta starts to undergo a secondary division at its anterior end, so that the point where it bifurcates is moved back from the level of the sixth aortic arch almost to the level of the coeliac artery (*Hughes, 1943*). On the right side, the newly differentiated segment of aorta posterior to the sixth arch divides into a dorsal branch continuing the fourth arch and a ventral branch continuing the sixth arch. The former branch persists, and, together with the right fourth arch, forms the permanent arch of the aorta. The aortal segments continuous with both sixth arches endure throughout incubation but atrophy when pulmonary respiration begins.

As Fig. 239 shows, the fate of the anterior dorsal aortae is not identical in all species of birds, for there is some variability in the adult configuration of the carotid arteries. In addition (*Meckel, 1826; Garrod, 1873*), a single right carotid artery is present in two species of bustard (*Otis*).

As for the ventral cephalic aorta, its unpaired portion very soon becomes indistinguishable from the truncus arteriosus of the heart, from which the third, fourth, and sixth pairs of aortic arches spring almost directly. With the disappearance of the first and second aortic arches, the paired ventral aortae remain, extending anteriorly as the transitory ventral carotid arteries. These participate in the formation of the external carotid arteries and the ascending and descending esophageal arteries.

By the technique of tissue culture, it has been shown that the aorta survives the exsanguination of the 7-, 10-, 15-, or 21-day chick embryo by 4 to 5 hours, at the most (*Grodzinski, 1932*).

### The Anterior and Posterior Cardinal Veins and the Duct of Cuvier

The formation of the cardinal veins in the chick embryo begins very shortly after the aortae start to differentiate and, in fact, can be traced back to the stage of 6 or 9 somites (*Williams, 1910; Sabin, 1917b*). At this time, a network of prevascular tissue extends dorsally from the aortae into the intersomitic spaces. This tissue is continuous anteriorly with the angioblastic primordia of the vena capitis medialis, which extend along each side of the rhombencephalon. By the 12-somite stage, the intersomitic strands of angioblasts have been converted into paired aortic branches between all the somites thus far present, and dilatations of the distal ends of the intersomitic arteries are anastomosing longitudinally to form the paired cardinal veins (*Sabin, 1917b*), lateral and parallel to the aortae. Cross-anastomoses in the first and second interspaces connect the cardinal veins with the primitive longitudinal veins of the rhombencephalon region.

At the 14-somite stage, each cardinal vein is in the form of a plexus opposite the second, third, and fourth somites, or at the level of the omphalomesenteric veins. A small vessel extending laterally from this plexus toward the omphalomesenteric vein represents the duct of Cuvier.



With the appearance of the duct of Cuvier, the cardinal vein can be differentiated into anterior and posterior divisions, which correspond, respectively, to the portions lying cephalic and caudal to the duct of Cuvier. The vena capitis medialis and its posterior portion (which was originally the transverse vein of the first interspace) are sometimes regarded as cephalic continuations of the anterior cardinal vein (Sabin, 1915; Evans, 1909b).

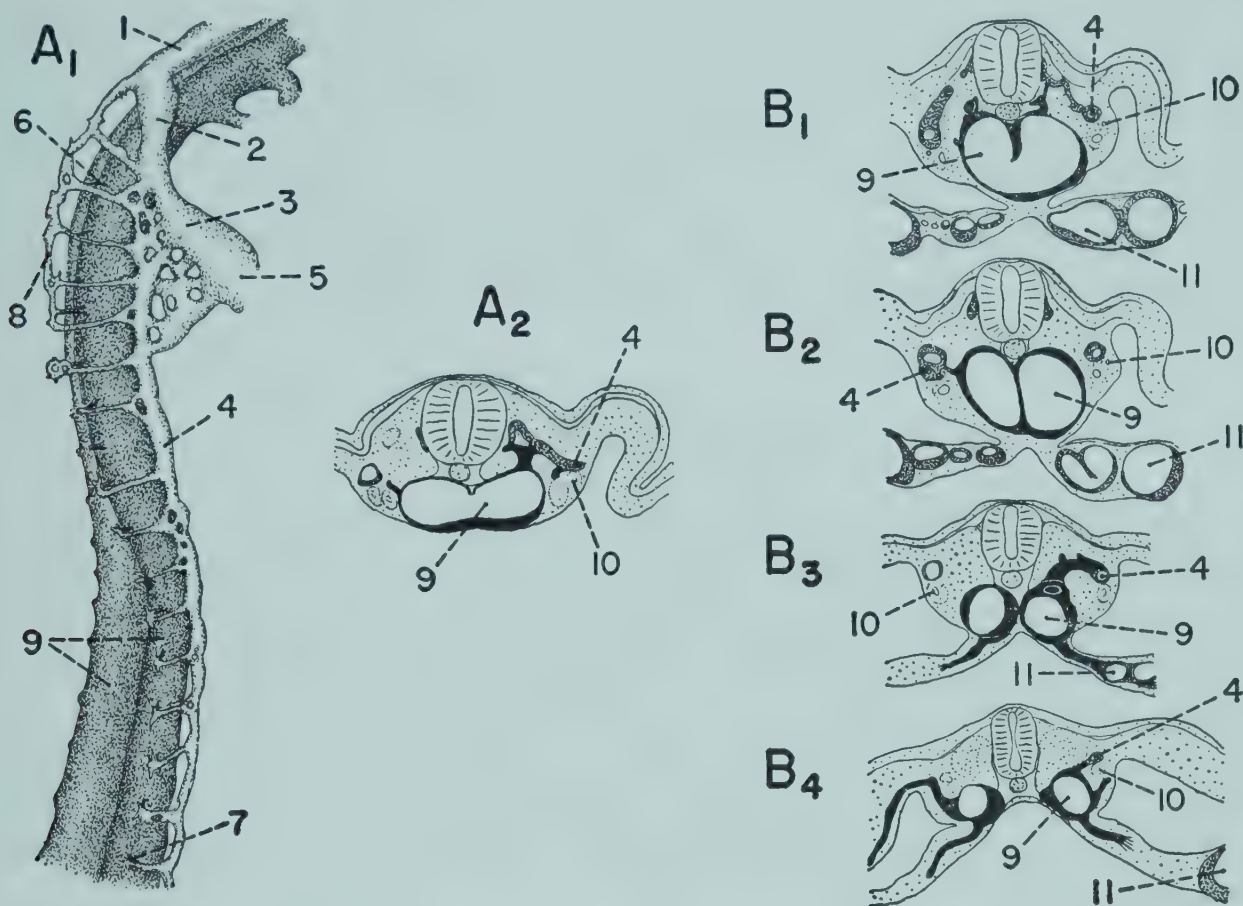


Fig. 240. Development of the spinal arteries, aorta, and the posterior cardinal vein in the 25- and 30-somite chick. (Redrawn with modifications after Sabin, 1917b.)

A<sub>1</sub>, at 25 somites; A<sub>2</sub>, transverse section at 25 somites; B<sub>1</sub>, transverse section through the fifteenth interspace at 30 somites; B<sub>2</sub>, same age through the sixteenth interspace; B<sub>3</sub>, same, through the twenty-first interspace; B<sub>4</sub>, same through the twenty-seventh interspace. All  $\times 40$ .

1, primitive vessel of the rhombencephalon (vena capitis medialis); 2, transverse vein of the first interspace; 3, anterior cardinal vein; 4, posterior cardinal vein; 5, duct of Cuvier; 6, artery of the third interspace; 7, artery of the eighteenth interspace; 8, plexus on the spinal cord; 9, dorsal aorta; 10, Wolffian duct; 11, omphalomesenteric vein.

The ducts of Cuvier form connections with the omphalomesenteric veins about at the time that circulation begins (the 16-somite stage). These connections complete the first purely intraembryonic blood circuit (Sabin, 1915).

The posterior cardinal veins reach the level of the hindlimb bud during the chick's third incubation day (Evans, 1909b) and are fully differentiated at the 30-somite stage (Hughes, 1934). As they extend posteriorly, the cardinal veins direct sprouts toward the spinal cord in each intersomitic space, and these segmental spinal veins anastomose with the segmental spinal arteries (Fig. 240-A<sub>1</sub> and B<sub>1</sub>).



The posterior cardinal veins and the ducts of Cuvier do not persist in their original form. The posterior cardinal veins participate in the formation of the system of the posterior vena cava (postcaval vein). The ducts of Cuvier are eventually incorporated into the superior venae cavae (pre-caval veins).

The anterior cardinal veins, which elongate at the expense of the posterior cardinal veins as the heart shifts caudad, eventually become the jugular veins. It has been experimentally demonstrated (*E.R. Clark, 1915*) that the conditions in the 2- to 6-day chick embryo's neck favor the development of a large vein in the position of the anterior cardinal vein. In embryos reincubated for 3 to 4 days after the unilateral removal of the anterior cardinal vein at the 48-hour stage, a large vein may be found on the operated side in the location normally occupied by the internal jugular vein.

### The Aortic Arches

The first or mandibular aortic arches, as previously noted, arise in the chick embryo at the 10-somite stage in the form of vascular loops connecting the dorsal and ventral aortae in the mandibular arch, anterior to the first visceral cleft (cf. Fig. 237-B<sub>1</sub>). These are the first of six pairs of aortic arches which appear in birds as in all amniotes. The aortic arches form in succession (*Balfour, 1873c*), from anterior to posterior, with the exception of the very transitory fifth pair, which is the last to make its appearance.

The second, third, and fourth aortic arches lie in the corresponding visceral arches, which they traverse in a direction perpendicular to the body axis. The first aortic arch is located well anterior to the first visceral cleft and lies obliquely; *Kastschenko (1887)* claimed that a temporary branch of the dorsal aorta which he found just behind the mandibular arch should be considered as the true first arch. The fifth and sixth aortic arches lie posterior to the fourth visceral pouch.

The time at which the aortic arches appear and disappear and their ultimate fate are indicated in Table 11. It may be noted in this table and in Fig. 241 that the first, second (hyoid), and fifth pairs of arches disappear entirely, as does also the left fourth arch, whereas the remaining arches or their branches persist as permanent parts of the definitive circulatory system. The first and second pairs break down into capillary plexuses and contribute to the formation of various vessels of the head (*Hughes, 1934*). When the dorsal aorta atrophies posterior to the third arch, the latter is added to the primitive internal carotid artery as a proximal segment. The obliteration of the left fourth arch is accompanied by the fusion of its root with the root of the left third (carotid) arch (*Mackay, 1888*), and, in some species, by the subsequent transforma-



tion of its remaining portion into a solid cord of cells (Mackay, 1888; Glenny, 1940). In the ostrich (*Struthio camelus*), the left fourth arch disappears at a relatively earlier period than in the chick (Fleming, 1926). In certain species, such as the belted kingfisher (*Ceryle alcyon*), the left fourth arch may remain patent and functional, though it loses its connection with the aortic root (Glenny, 1940).

TABLE 11

## Appearance and Disposition of the Aortic Arches in the Developing Chick Embryo

Arch No.	Name	Time of Appearance (incubation day)	Ultimate Fate
I	Mandibular	2	Plexiform before third day; disappears on third to fourth day <sup>1,2,9,11</sup>
II	Hyoid	2-3	Plexiform on fourth day; disappears on fourth to fifth day <sup>1,9,11</sup>
III	Carotid	2-3	Carotid arteries take origin from the arch <sup>14</sup> Secondary subclavian arteries originate from the ventral portion of right and left arches <sup>6,12</sup>
IV	Aortic or systemic	3	Left arch becomes obliterated and finally disappears during the sixth or seventh day; right arch persists as permanent arch of the aorta <sup>2,3,4,5,6,7,8,9,10,11,13</sup>
V	(Temporary)	4-5	Disappears by sixth day <sup>1</sup>
VI	Pulmonary	3.5-4	Each arch functions as ductus arteriosus connecting aorta and pulmonary artery; one pulmonary artery branches from each arch and becomes its main stem, and the ductus arteriosus disappears <sup>4,5,6,9,12</sup>

<sup>1</sup> Baer (1827); <sup>2</sup> Baer (1828a); <sup>3</sup> Bakst and Chafee (1928); <sup>4</sup> Bemmelen (1886); <sup>5</sup> Boas (1887); <sup>6</sup> Bremer (1928b); <sup>7</sup> Fleming (1926); <sup>8</sup> Foster and Balfour (1893); <sup>9</sup> Kastschenko (1887); <sup>10</sup> Locy (1906); <sup>11</sup> Mackay (1888); <sup>12</sup> Mall (1887); <sup>13</sup> Rathke (1857); <sup>14</sup> Twining (1906).

The fifth aortic arches are offshoots of the sixth (Locy, 1906) and seem to be merely rudiments, of phylogenetic significance only. The existence of the fifth aortic arch in birds remained unrecognized for many years, and the sixth arch was regarded as the fifth. The fifth arch, first identified by Bemmelen (1886) and Locy (1906), is present only briefly after its appearance on the chick's fourth to fifth day of incubation. Locy (1906) found the fifth aortic arch to be lacking on one side in some chick embryos, al-



though it is possible that this seeming asymmetry was observed merely because development was not synchronous on the two sides.

The sixth arch becomes complete in the chick of about 35 somites when an angioblastic strand growing down from the angle between the fourth aortic arch and the dorsal aorta unites with one growing upward from the ventral aorta (Buell, 1922). Once junction is made, a rapid increase in the size of the arch ensues.

The ventral or proximal portion of the sixth aortic arch persists permanently as the proximal portion of the pulmonary artery, which is given off from the sixth arch. The dorsal portion of the arch, above the root of the pulmonary artery, is known as the ductus arteriosus. After the chick's sixth incubation day, when the dorsal aorta starts to undergo a secondary divi-

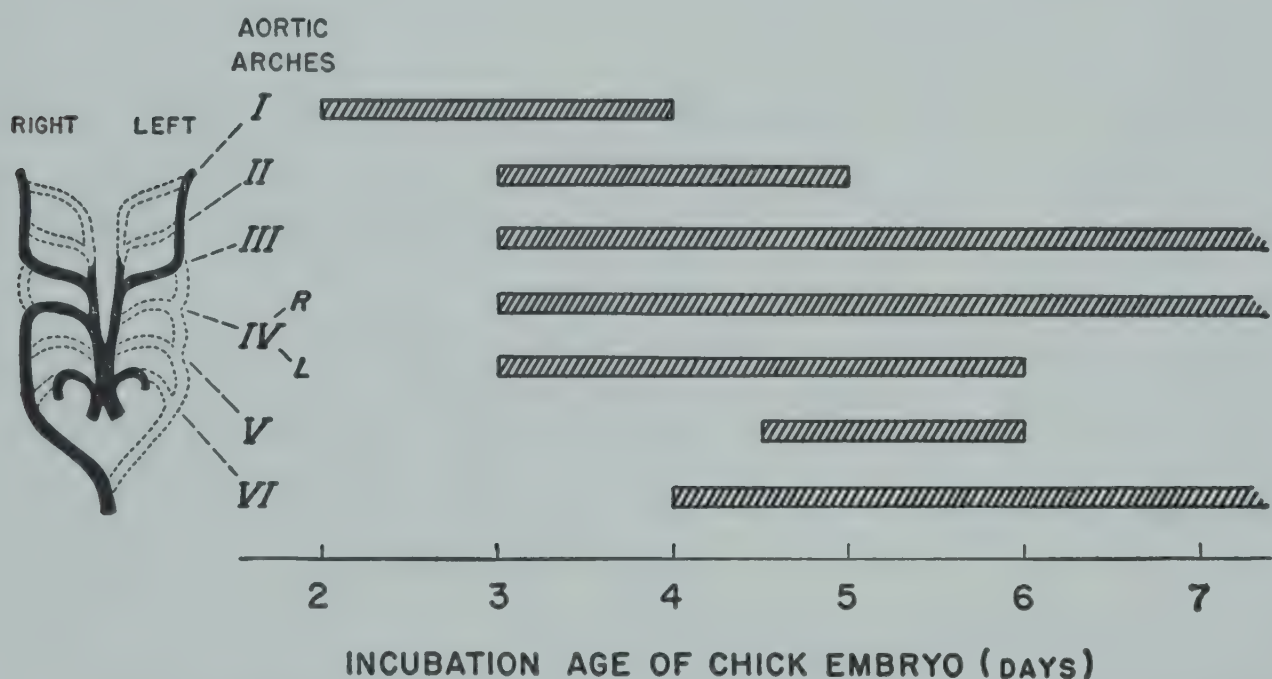


Fig. 241. Diagram of the persistent aortic arches in birds, and a graph of the periods during which each of the six aortic arches is present in the chick embryo. (Compiled by the author from various sources.)

Note that of the fourth pair, only the right aortic arch persists in Aves.

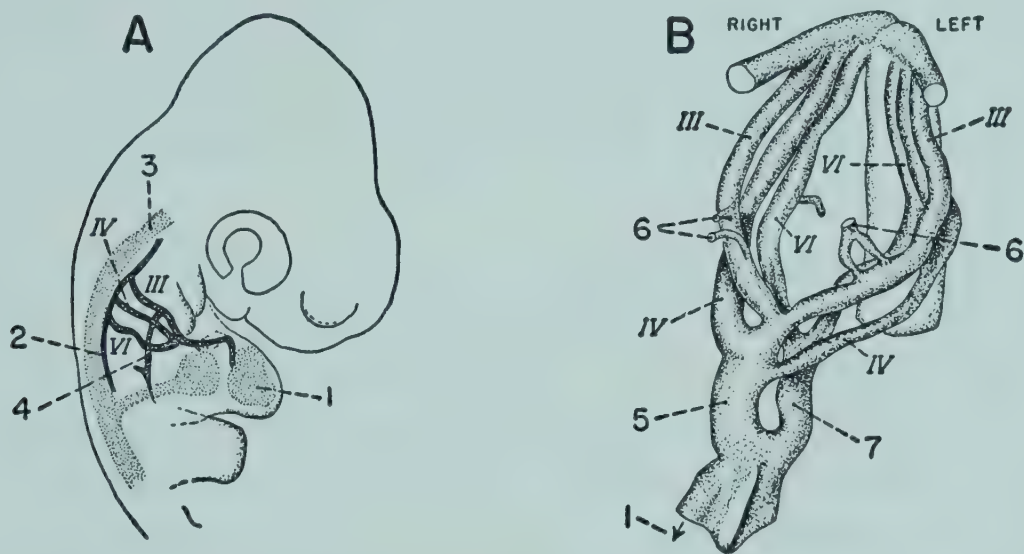
sion anteriorly, the ductus arteriosus is lengthened by the addition of a newly differentiated segment of dorsal aorta. The entire ductus arteriosus becomes a solid cord of tissue shortly after the onset of pulmonary respiration. The contraction of the lumen of the ductus begins proximally and progresses distally (Hughes, 1943).

The disappearance of some aortic arches and the persistence of others are largely the results of hemodynamic conditions and provide evidence that the blood circulation is a factor that determines the course of vessels within the embryo, much as it does in the area vasculosa (Thoma, 1893). The persistence of the permanent aortic arches is directly dependent upon the existence of a dilating force exerted by a strong blood stream. The aortic arches that normally disappear (especially those on the left side) can be made to persist if those on the opposite side are ligated (Stephan, 1949;



Wolff and Stephan, 1948a) or destroyed by ultraviolet point radiation of their roots for 45 seconds (Hinrichs, 1931). The right fourth arch, for example, can be supplanted by the left fourth arch or by one of the third pair; and the common carotid arteries (normally springing from the third arches) become associated with whatever surviving arches are most anterior, or with the dorsal aorta, if all aortic arches are obliterated (Stephan, 1949).

The effect of mechanical factors on the aortic arches becomes apparent at the beginning of the chick's third incubation day (Kranichfeld, 1914). At this time, the primitive truncus arteriosus of the heart starts to elongate, and, like all lengthening vessels (Hughes, 1937), it grows narrower. Consequently, the mouths of the first and second aortic arches are constricted, since these arches are given off directly from the truncus. The second



**Fig. 242.** Location of the aortic arches present in the chick embryo. (Redrawn with modifications A, after Mackay, 1888; B, after Bremer, 1928b.)

A, at the beginning of the fifth incubation day; B, the relationship of the aortic arches to each other and to the dorsal and ventral aortae.

1, heart; 2, dorsal aorta; 3, anterior cardinal vein; 4, secondary subclavian artery; 5, aortic trunk; 6, severed remnants of ventral aorta; 7, pulmonic trunk.

aortic arch on the left side is constricted sooner than its counterpart on the right side, because the axis of the truncus is now inclined in such a way that the main blood stream is directed to the right. By the middle of the third day, the mouths of the first and second arches are reduced to slits, and most of the blood from the heart is directed into the third aortic arches. The disappearance of the first and second arches follows during the fourth day. The left third aortic arch, receiving less blood than the right, is slightly narrowed; but no further hemodynamic effect occurs, for the truncus arteriosus is now a long tube that makes a gradual transition to the third aortic arches (Kranichfeld, 1914). Between the 4-day and 7.5-day stages, the heart and aortic arches are migrating backward (Hughes, 1934). The torsion and elongation of the truncus arteriosus caused by the "descent" of the heart into the thorax are thought to be the chief factors responsible for the obliteration of the left fourth aortic arch. This arch is



apparently bent, displaced, and stretched more than the right arch. As the left arch becomes narrower, the right receives more blood, especially since it is rotated (Fig. 242-B) into a position almost directly in line with the truncus arteriosus (Bremer, 1928b). The left fourth arch is said to be placed at an additional disadvantage because the bending caused by rotation is intensified by pressure against the body wall and the pulmonary arch (Congdon and Wang, 1926). By the 6.5-day stage, when the aortic arches have moved posteriorly through twenty segments (Hughes, 1934), the lumen of the left fourth arch has been reduced to capillary size and is clogged with blood cells. After the lumen is completely occluded, degeneration of the wall starts in the two or three layers of cells which are applied to the outer surface of the endothelium (Hughes, 1943).

Spontaneous contractions (at the rate of five to twenty per minute) have been observed in explanted fragments of aortic arches and caudal aortae from 5- to 20-day chick embryos (Attardi, Gandini, and Marcon, 1948). Independent pulsation of arteries *in vivo* is thought not to occur in vertebrates, however.

**Histogenesis of the Aortic Arches.** In general, the aortic arches of the chicken are elastic arteries differing in structure from the peripheral muscular arteries. Portions of two of the aortic arches are of a third type, which is usually intermediate in position and structure between the elastic and muscular arteries. The elastic arteries have the thickest walls, consisting chiefly of a middle zone, or tunica media, in which layers of muscle and elastic fibers alternate. The outer muscle layers are longitudinal and the inner ones are obliquely transverse. There is also an inner intimal zone containing longitudinal elastic fibers, and a thin outer or adventitial zone containing collagen fibers. In the muscular arteries, the muscles, which run circumferentially, are isolated in the inner half of the tunica media, the outer half of which consists of several layers of elastic fiber networks. The intima is replaced by a thin elastic fiber network, and the adventitia is very thick. The intermediate type of artery also has a thick adventitia and an inner elastic network, but the tunica media consists of alternate muscle and elastic layers of fibers which, as in the muscular arteries, run circumferentially (Hughes, 1943).

At first, the aortic arches consist of a single layer of endothelial cells. The histogenesis of the arches, according to the account given by Hughes (1943), begins with the flattening of a layer of mesenchyme cells against the outer surface of the endothelium. Other layers added outside this layer form a compact zone which represents the future tunica media. In the 5-day chick embryo, the aortic arches which will persist throughout incubation have acquired two or three layers of cells superficial to their endothelium. Between the fifth and ninth days, the thickness of the tunica media increases more rapidly in the proximal part of the pulmonary arch



than in the other arches, but thereafter the carotid and right fourth (systemic) arches lead in development. At the time of hatching, the tunica media is made up of about twenty-five layers of cells in the pulmonary arch, as compared with forty in the carotid arch and fifty or more in the systemic arch.

The distal portion of the pulmonary arch, the ductus arteriosus, differentiates not as an elastic artery but as an artery of the intermediate type, and the difference between its structure and that of the proximal portion of the pulmonary arch is apparent at the 9-day stage. Immediately distal to the root of the pulmonary artery, the cell layers decrease in number and are very compactly arranged. In this region, which is the first part of the ductus arteriosus to degenerate, the cell layers of the tunica media remain fewer in number and more closely compressed than in the distal part, where the number of layers reaches its maximum of only twelve to fourteen by the 13-day stage. At that time, the adventitia of the ductus arteriosus consists of four cell layers with collagen bundles between them.

The systemic arch also exhibits the structure of an elastic artery proximally and that of an intermediate type of artery distally. The transition is made more gradually than in the pulmonary arch and occurs at a continually more distal point as incubation proceeds. On the thirteenth day, the tunica media consists of about forty cell layers proximally and of about fifteen cell layers distally. At hatching, a short segment of the arch immediately proximal to its junction with the dorsal aorta is the only portion which exhibits the intermediate arterial structure.

In the aortic arches of the 7.5-day chick, the cells of the future tunica media are arranged in layers (corresponding to the muscle layers), between which are clear spaces. All the cells of the muscle layers are present in the pulmonary arch by this stage, but the full complement is not reached in the systemic and carotid arches until the 11-day stage. Subsequent additions are made to the intimal zone and to the future elastic layers between the muscle layers. At the 13-day stage, the cells of all elastic layers are present between the muscle layers of the systemic arch but are just beginning to appear in the pulmonary arch. An intima consisting of two cell layers can be identified in the carotid and systemic arches at 7.5 days but does not appear in the pulmonary arch until the eleventh day, and, at 21 days, the intimal zone of the pulmonary arch is only half as thick as that of the systemic arch.

The smooth muscle fibers begin to differentiate in the aortic arches on the ninth or tenth day. The ratio of the length of the myoblast nuclei to their length increases from 2:1 on the ninth day to 4:1 on the sixteenth day. The first muscle fibers to differentiate from myoblasts appear on the ninth or tenth day. Their nuclei are much longer and narrower than those



of fibers differentiating later. Fibers of the early type are still present in abundance on the sixteenth day but have degenerated by the twenty-first day. At the 7.5-day stage, the outermost myoblast nuclei tend to be oriented longitudinally. At the 9-day stage, the nuclei in the deeper layers are aligned more or less transversely, and thus the definitive orientations are indicated. The innermost nuclei, however, run longitudinally, as do those of the intimal zone, which, therefore, is not clearly demarcated. A week later, there are only a few longitudinal muscle fibers near the intima, and these subsequently disappear.

Elastic fibers first appear at the 7-day stage among the outermost mesenchyme cells of the aortic arch walls. At the 7.5-day stage, a few fibers may be seen between the future muscle layers. After 9 days' incubation there are networks of elastic fibers between all the myoblast layers and in the intimal zone, where the fibers are finest and where they run longitudinally. At 11 days, elastic fibers of another type are seen, closely applied to the surfaces of muscle layers. Fibers covering two adjacent muscle layers are linked together by bundles of coarser elastic fibers. Subsequently, the fibers on the muscle layers, as well as the connecting bundles, become much heavier and more conspicuous, and the finer network is reduced in importance.

In general, the collagen fibers in the adventitia develop after the sixteenth day.

### The Principal Vessels of the Head and Neck

The development of the vascular system of the head and neck regions is characterized by the formation of many primitive vessels and their replacement by others, before the final arterial and venous channels are established. In the chick, the arteries of these regions approximate the definitive pattern by the ninth day, or somewhat earlier than the veins. This pattern is influenced to a large extent by the backward migration of the heart and the aortic arches, which affects the subclavian, vertebral, and external carotid arteries especially (*Hughes, 1934*). Also, it is evident that many arteries, veins, and nerves develop along the same paths.

#### *The Arteries of the Head and Neck*

All the arteries of the head are branches of the carotid arteries and thus are essentially derivatives of the dorsal and ventral cephalic aortae. The arteries of the neck originate from the dorsal aortae and the segmental arteries.

In the chick embryo of 32 somites (*Hughes, 1934*), the dorsal aorta has already grown out as the internal carotid artery for some distance forward of the level of the first aortic arch, which has by now become plexiform. At the base of the forebrain, the internal carotid artery divides into the



caudal and cranial rami of the circle of Willis (Fig. 243-A). The caudal ramus gives off the bigeminal artery to the optic lobes and then turns sharply backward to run along the ventral surface of the hindbrain as the basilar artery, which anastomoses with the most anterior intersomitic arteries. The cranial ramus extends ventrolaterally along the entire forebrain, giving off the ophthalmic cerebral artery anterior to the optic vesicle. Posterior to the optic vesicle is the ophthalmic ramus of the internal carotid, given off just caudal to the point of origin of the cranial ramus of the circle of Willis. The ophthalmic ramus and the ophthalmic cerebral artery anastomose with each other by the 4.5-day stage, at which time the basilar arteries have fused into a single median vessel as far caudally as the vagus nerve.

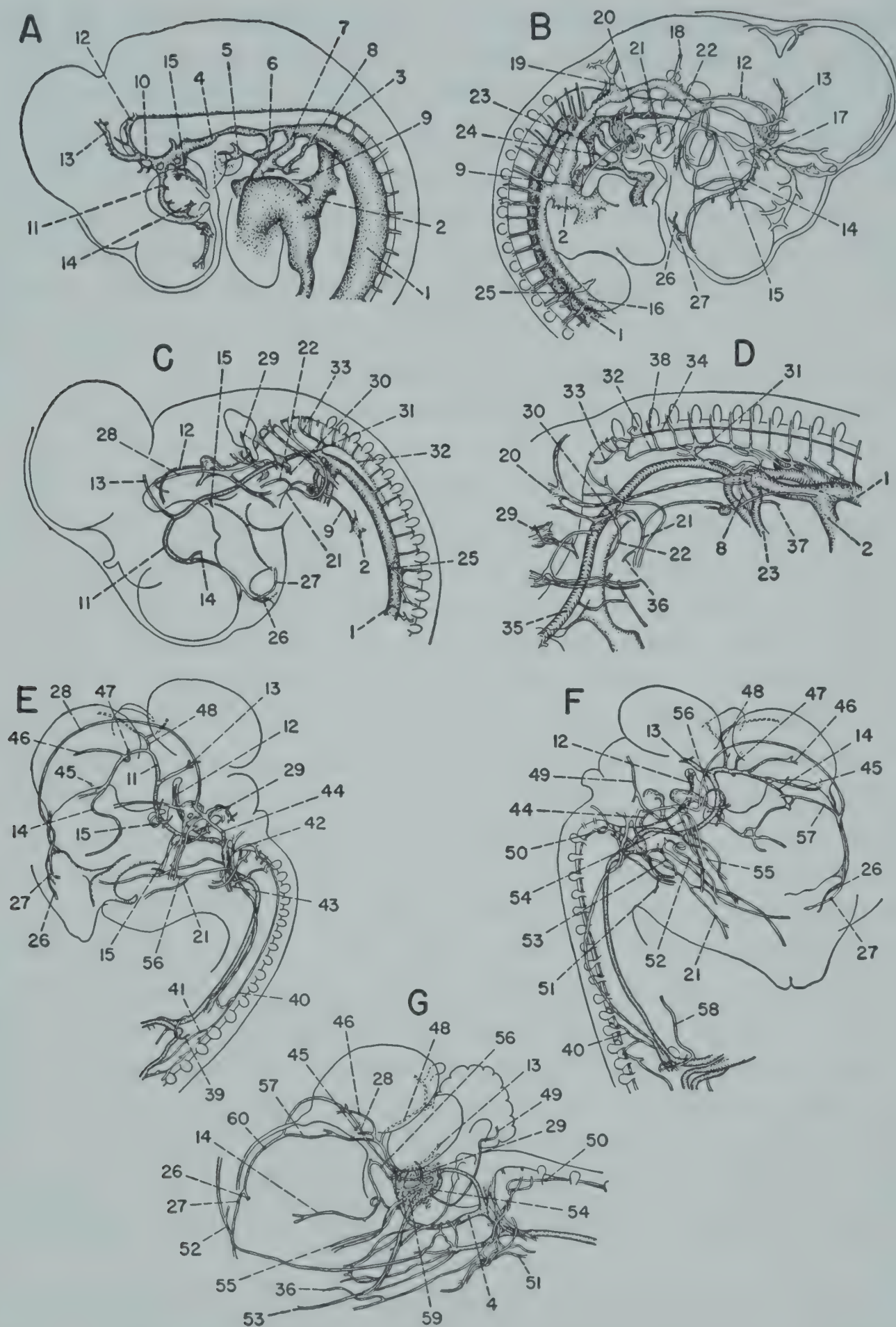
Meantime, at the 4-day stage, the plexus in the mandibular arch has sent out a single capillary into the maxillary region. In the 4.5-day embryo, the ventral aorta (which extends anteriorly from the base of the third aortic arch) ends in two branches, a maxillary ramus and a mandibular ramus (Fig. 243-B); the ventral aorta may now be considered as the ventral (primitive external) carotid artery (*Twining, 1906; Hughes, 1934*).

At the 4.5-day stage, the plexus of the hyoid arch is connected with the dorsal aorta by a small vessel which is to form the root of the stapedial artery. Twenty-four hours later (5.5 days), a small branch of the internal carotid artery can be seen to arise very close behind the root of the stapedial artery (*Twining, 1906; Hughes, 1934*). This small spur is the root of the definitive external carotid artery (cf. Fig. 243-C).

It has been proposed (*Fleming, 1926*) that the root of the definitive external carotid artery arises from a point near the dorsal end of the third aortic arch in both the chick and the ostrich (*Struthio camelus*) and grows forward to reach its final position (cf. Fig. 245; Fig. 252). These findings could not be corroborated by Hughes (1934). Hafferl (1921) suggested that the root of the external carotid artery of the European lapwing (*Vanellus vanellus*) is a ventral branch of a segmental artery, but otherwise his account is in good agreement with that of Hughes (1934).

At the 6.5-day stage (cf. Fig. 243-D), the root of the definitive external carotid artery has anastomosed with the ventral carotid artery (*Twining, 1906*) at the point where the latter divides into maxillary and mandibular rami (*Hughes, 1934*). Immediately before making the anastomosis, the external carotid artery gives off a capillary that runs upward and backward and represents the occipital artery. The ventral carotid artery is no longer able to keep pace with the elongation of the region in which it lies, and, by the 7.5-day stage (cf. Fig. 243-E), it ruptures in its midportion (*Twining, 1906*). The anterior portion becomes the descending esophageal artery, branching from the mandibular ramus of the external carotid, and





**Fig. 243.** Development of the arteries in the head and neck of the chick embryo. (Redrawn with rearrangement after Hughes, 1934.)

A, at 2.5 days' incubation ( $\times 10$ ); B, at 4.5 days ( $\times 8$ ); C, at 5.5 days ( $\times 6$ ); D, at 6.5 days ( $\times 8$ ); E, at 7.5 days ( $\times 5$ ); F, at 9 days ( $\times 4$ ); G, at 21 days ( $\times 2$ ).

1, dorsal aorta; 2, duct of Cuvier; 3, artery of the second interspace; 4, internal carotid artery; 5, superficial plexus of the mandibular arch; 6, second aortic arch; 7, third aortic arch; 8, fourth aortic arch; 9, secondary subclavian artery; 10, caudal ramus of the circle of Willis; 11, cranial ramus of the circle of Willis; 12, basilar artery; 13, bigeminal artery; 14, ophthalmic cerebral artery; 15, ophthalmic branch of the internal carotid artery; 16, posterior cardinal vein; 17, anterior cerebral vein; 18, middle cerebral vein; 19, pos-



the posterior portion becomes the ascending esophageal artery, springing from the base of the third aortic arch (*Hughes, 1934*). By the 7.5- ( *Hughes, 1934* ), or 8.5-day stage (*Twining, 1906* ), the dorsal aorta has disappeared between the third and fourth right arches, and the left fourth arch has atrophied. Posterior to the root of the external carotid artery, the original dorsal aorta is now known as the common carotid artery as far proximally as the point on the third aortic arch where the secondary subclavian artery is given off.

All the main branches of the external carotid artery can be recognized in the 9-day chick embryo, for the hyoid artery (later to supply the distal part of the geniohyoid muscle) has appeared between the occipital artery and the mandibular ramus (cf. Fig. 243-*F*). In the 12-day embryo (Fig. 244), the branches of the external carotid artery have attained practically their definitive arrangement. The occipital artery gives off, distally, a lateral branch to the neck muscles and a median branch that is the anterior root of the vertebral artery. Near its base, the occipital artery gives off a median branch and a posterior branch; the latter is the vagus artery. From the mandibular ramus of the external carotid artery there arise the superior laryngeal artery, passing to the larynx; the descending esophageal artery; and, more distally, the lingual artery, passing to the tongue. The maxillary ramus extends for a short distance beyond the mandibular ramus and then divides into five smaller arteries. Two of these, the auricular and the external facial, extend upward; the latter sends a branch to the nasal and frontal regions. The remaining three are the upper, middle, and lower maxillary arteries. The middle maxillary artery, which represents the earlier maxillary ramus of the external carotid artery, receives the sphenomaxillary branch of the internal carotid, the anastomosis having been established at the 6.5-day stage (*Hughes, 1934*).

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terior cerebral vein; 20, root of stapedial artery; 21, mandibular ramus of the external carotid artery; 22, maxillary ramus of same; 23, sixth aortic arch; 24, fifth aortic arch; 25, primary subclavian artery; 26, internal ethmoid artery; 27, external ethmoid artery; 28, supraorbital ramus of the stapedial artery; 29, temporal ramus of same; 30, root of secondary external carotid artery; 31, communal cervical artery; 32, primary sympathetic plexus; 33, artery of the fourth interspace; 34, secondary sympathetic plexus; 35, sphenomaxillary artery; 36, lingual artery; 37, pulmonary artery; 38, vein of eighth interspace; 39, definitive secondary subclavian artery; 40, vertebral artery; 41, artery of nineteenth interspace; 42, occipital artery; 43, jugular vein; 44, stapedial artery; 45, anterior cerebral artery; 46, middle cerebral artery; 47, branch of middle cerebral artery to the chorioid plexus; 48, posterior cerebral artery; 49, cerebellar artery; 50, occipital artery forming root of the vertebral artery; 51, descending esophageal artery; 52, external facial artery; 53, lower internal maxillary artery; 54, inferior alveolar ramus of the stapedial artery; 55, infraorbital ramus of same; 56, ophthalmic ramus of same; 57, anastomosis between cerebral ophthalmic and ethmoid arteries; 58, ascending esophageal artery; 59, temporal rete; 60, ethmoid artery.



To return now to the chief branches of the internal carotid artery: these are almost all present by the 7.5-day stage (cf. Fig. 243-E). Between the roots of the bigeminal and the ophthalmic cerebral arteries, the cranial ramus of the circle of Willis gives off the posterior, middle, and anterior cerebral arteries. The posterior cerebral artery has an anterior and a posterior ramus. The middle cerebral artery, which runs forward along the lateral surface of the cerebral hemisphere, gives off a branch that will supply the anterior chorioid plexus. The cerebellar artery, which supplies the posterior chorioid plexus, has appeared as a branch of the basilar artery and runs obliquely upward behind the root of the acoustico-facialis nerve.

Meantime, at the 5.5-day stage (cf. Fig. 243-C), the ethmoidal artery (the anterior continuation of the cranial ramus of the circle of Willis) has divided distally into a lateral and a medial vessel, the external and internal ethmoidal arteries, respectively. At the 7.5-day stage, the proximal end of the ethmoidal artery has lost its connection with the cranial ramus of the circle of Willis and has become continuous with the supraorbital ramus of the stapedia artery.

The stapedia artery is the most important branch of the internal carotid artery. As previously mentioned, its root is seen at the 4.5-day stage connecting the plexus of the hyoid arch with the dorsal aorta. A similar origin in the European lapwing (*Vanellus vanellus*) has been described by Hafferl (1921). The stapedia root is also connected with the lateral head vein, but this connecting capillary has been superseded a day later by a capillary that runs forward, giving off a branch (the temporal ramus) to a plexus over the otocyst. Farther forward, this capillary, which is the stapedia artery, passes through a large plexus (the forerunner of the temporal rete) around the maxillomandibular root of the trigeminal nerve, continues as the supraorbital ramus of the stapedia artery, and ends in a plexus over the orbit. It is this ramus which has connected with the ethmoidal artery at the 7.5-day stage, the elongation of the interorbital septum having caused the breaking of the original connection of the ethmoidal artery. The supraorbital ramus follows a semicircular course over the orbit to the ethmoidal region.

At the 9-day stage (cf. Fig. 243-F), the ethmoidal artery has established a new connection with the ophthalmic cerebral artery. As the main stem of the stapedia leaves the temporal rete, it gives off its own ophthalmic ramus which, after a short course, unites with the ophthalmic ramus of the internal carotid; the latter in turn has anastomosed with the distal part of the ophthalmic cerebral. The ventrally directed inferior alveolar branch of the stapedia, differentiated at the 7.5-day stage, now gives off the infraorbital ramus to the lower eyelid.

When (by the 12-day stage) the proximal part of the ophthalmic



cerebral artery has disappeared, its distal part remains as a continuation of the ophthalmic ramus of the internal carotid. The cross-connection between the ethmoidal and ophthalmic cerebral arteries remains permanently as a connection between the middle cerebral and ethmoidal arteries.

As previously mentioned, a branch of the occipital artery becomes the anterior root of the cervical vertebral artery. In both the chick and the ostrich, *Struthio camelus* (Fleming, 1926), the formation of the cervical vertebral artery is directly affected by the backward migration of the heart and the aortic arches. In the chick, the aortic arches have moved back-

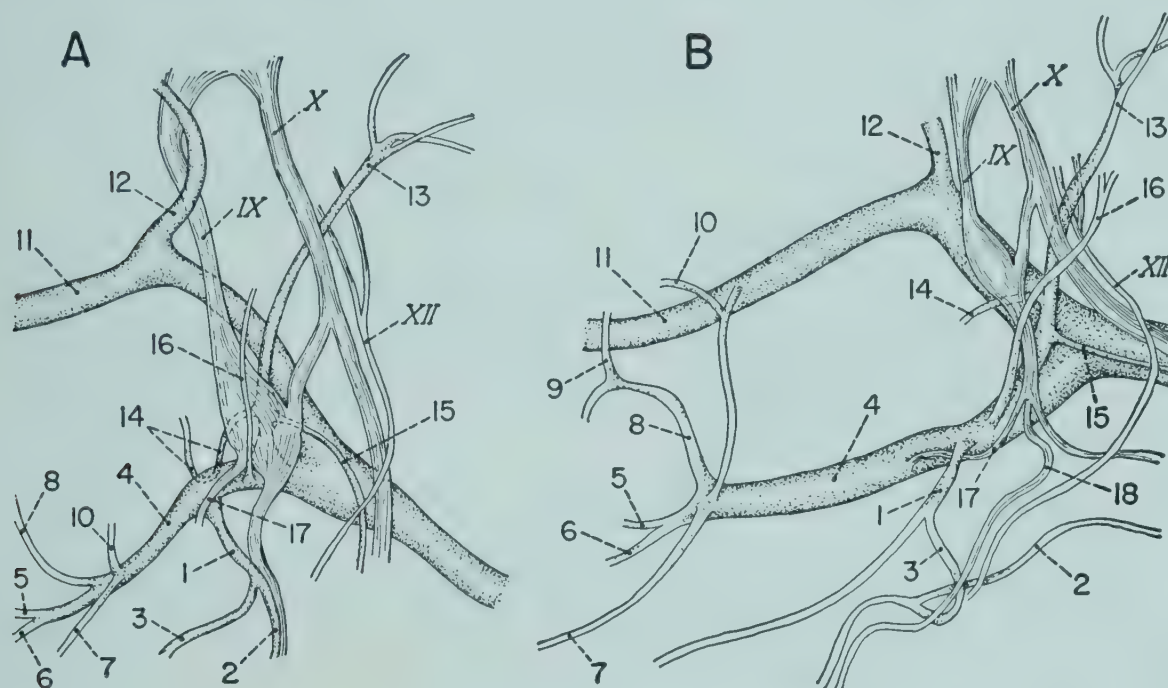


Fig. 244. Details of the external carotid artery of the chick embryo. (Redrawn with modifications after Hughes, 1934.)

A, at 12-day stage ( $\times 20$ ); B, at 21-day stage ( $\times 10$ ).

1, mandibular ramus of external carotid artery; 2, descending esophageal artery; 3, superior laryngeal artery; 4, maxillary ramus of external carotid artery; 5, 6, 7, upper, middle, and lower internal maxillary arteries, respectively; 8, external facial artery; 9, ascending branch of external facial artery; 10, auricular artery; 11, internal carotid artery; 12, stapedial artery; 13, occipital artery; 14, anterior median branch of occipital artery; 15, definitive root of vertebral artery; 16, hyoid artery; 17, glossopharyngeal nerve to geniohyoid muscle; 18, laryngeal branch of glossopharyngeal nerve; IX, X, XII, glossopharyngeal, vagus, and hypoglossal nerves, respectively.

ward past six segments after 5.5 days' incubation (Hughes, 1934), and the roots of the six most anterior intersomitic arteries have been so greatly displaced that they have overlapped and fused into a single stem (Fig. 245-B; Fig. 243-C). This stem has been called the common cervical artery (Fleming, 1926). Fleming (1926) believed that the common cervical artery of the ostrich (*Struthio camelus*) consisted of the enlarged first intersomitic artery (cf. Fig. 245-A). At the time this artery forms, several intersomitic arteries below it have lost their connections with the dorsal aorta. Distally, all these intersomitic arteries run into a plexus investing the primary sympathetic ganglionic chain. By the chick's 6.5-day stage (cf.



Fig. 243-D), the secondary sympathetic chain has appeared dorsal to the primary chain and has also acquired an investing plexus. The plexus of the primary chain then starts to degenerate, and it has disappeared by the 7.5-day stage. The cervical vertebral artery now resolves (cf. Fig. 243-E) from the plexus of the secondary sympathetic chain (Hughes, 1934). Its formation has also been described as resulting from longitudinal anastomoses between the distal ends of the intersomitic arteries, the anastomoses being preceded by rather unusual terminal enlargements of the arteries (Krassnig, 1913b; Fleming, 1926). At this stage, the intersegmental arteries have severed their connections with the dorsal aorta (now the common carotid artery, in this region) as far down as the root of the primary subclavian artery, which has now joined with either the fourteenth

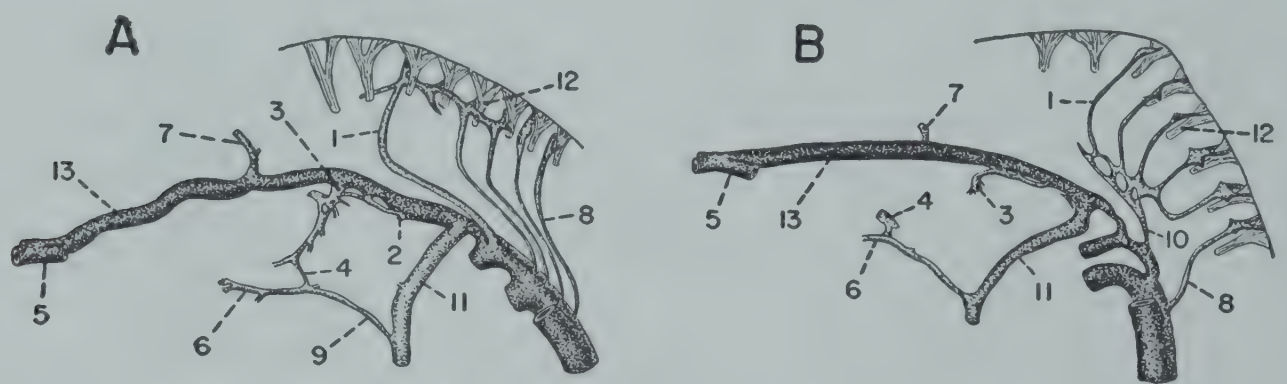


Fig. 245. Comparative development of the vertebral artery in embryos of the chick, *Gallus gallus*, and the ostrich, *Struthio camelus*. (Redrawn after Fleming, 1926.)

A, an early stage in the formation of the vertebral artery of the 13.5-mm. ostrich embryo; also shown is the shifting of the dorsal external carotid artery from the top of the third aortic arch to a secondary position along the course of the dorsal carotid artery; B, the corresponding stage of development of the vertebral artery of the chick embryo (10 mm.)

1, first spinal dorsal segmental artery; 2, primary dorsal external carotid artery; 3, secondary dorsal external carotid artery; 4, primary inferior alveolar artery; 5, cephalic sinus; 6, lingual artery; 7, stapedial artery; 8, sixth spinal dorsal segmental artery; 9, ventral (external) artery; 10, arteria cervicis communis; 11, third aortic arch; 12, third spinal nerve; 13, dorsal carotid artery.

(Krassnig, 1913b), sixteenth (Fleming, 1926), or eighteenth (Hughes, 1934) intersomitic artery, or possibly with the twentieth, in the ostrich, *Struthio camelus* (Fleming, 1926). The figures of Twining (1906) imply that the common cervical artery persists as the carotid root of the vertebral artery. More probably, however, the common cervical artery either disappears, leaving part of the primary subclavian artery as the carotid root of the vertebral artery (Fleming, 1926); or else it joins with the persistent eighteenth intersegmental artery—the proximal portion of the carotid root of the vertebral artery thus being formed of the common cervical artery and the distal portion being provided by the intersegmental artery (Hughes, 1934).

According to Hughes (1934), the anterior connection of the cervical



vertebral artery with the occipital artery does not form until the chick's ninth day, although Fleming (1926) stated that it is established after 5.5 days of incubation. In the ostrich (*Struthio camelus*), the anterior root is merely a direct, secondary anastomosis between the common carotid artery and the distal portion of the common cervical artery (cf. Fig. 252-C); the proximal portion of the latter atrophies (Fleming, 1926). Hafferl (1921) stated that the vertebral arteries of the lapwing (*Vanellus vanellus*) continue anteriorly into two small vessels that originate by bifurcation of the basilar artery, and that the first cervical arteries join these vessels rather than the vertebral arteries.

It has been said (Krassnig, 1913b; Fleming, 1926), although not without dispute (Hughes, 1934), that a third connection between the cervical vertebral and common carotid arteries is formed at the level of the fifth or eighth spinal nerve by the end of the ninth incubation day. Twining (1906) reported that the ascending cervical artery and the "arteria colli collateralis" are given off anteriorly and posteriorly, respectively, from the root of the vertebral artery in chick embryos of more than 8.5 days' incubation.

**Histogenesis of the Carotid Artery.** In the hatched chick, the carotid artery varies in histological structure throughout its course. Its proximal portion, derived from the third aortic arch, is a typical elastic artery. A short distance anterior to the root of the subclavian artery, the carotid becomes an artery of the intermediate type, and then, after giving off the vertebral artery, rapidly assumes the characteristics of a peripheral muscular artery. A short segment of the common carotid immediately proximal to its division into internal and external branches again exhibits the structure of an elastic artery.

The histogenesis of these segments of the carotid artery has been described by Hughes (1943). The elastic type of structure characterizing the carotid arch region extends only as far as the subclavian artery at the 9-day stage but is recognizable somewhat farther anteriorly at the 11-day stage; the limits of this structural zone are now the same as at hatching. The other two structural regions are also apparent at 11 days. The region of intermediate arterial structure is clearly indicated by the regular alternation of well-developed elastic laminae and cell layers throughout most of the tunica media. Anterior to the vertebral artery, the elastic fibers are better developed in the outer portion of the tunica media than in the inner portion. This difference is more pronounced at the 13-day stage, when the separation of the tunica media into an inner muscular and an outer elastic zone has progressed farther. Near the bifurcation of the carotid, the number of cell layers undergoes an increase in number from seven to twenty-four between the tenth and the fourteenth days, and is further augmented thereafter. In the 14-day embryo, the transition from



the intermediate to the muscular type of arterial structure is clearly seen as, in passing anteriorly, alternating muscular and elastic layers gradually replace the distinct and separate muscular and elastic zones and an intimal zone makes its appearance. The cell layers of the adventitial zone are identifiable at the 11-day stage along the entire course of the carotid artery but are progressively more obvious from the proximal to the distal end. Elastic and collagen fibers appear in the adventitia at 13 days. After this time, the adventitia increases steadily in thickness, except in the region of the bifurcation of the artery.

### *The Veins of the Head and Neck*

As previously noted, the anterior cardinal vein, the transverse vein of the first interspace, and the primitive vena capitis medialis differentiate in the chick embryo between the 6-somite and the 14-somite stage, or only slightly less precociously than the aortae. These veins are the primitive veins of the head and neck.

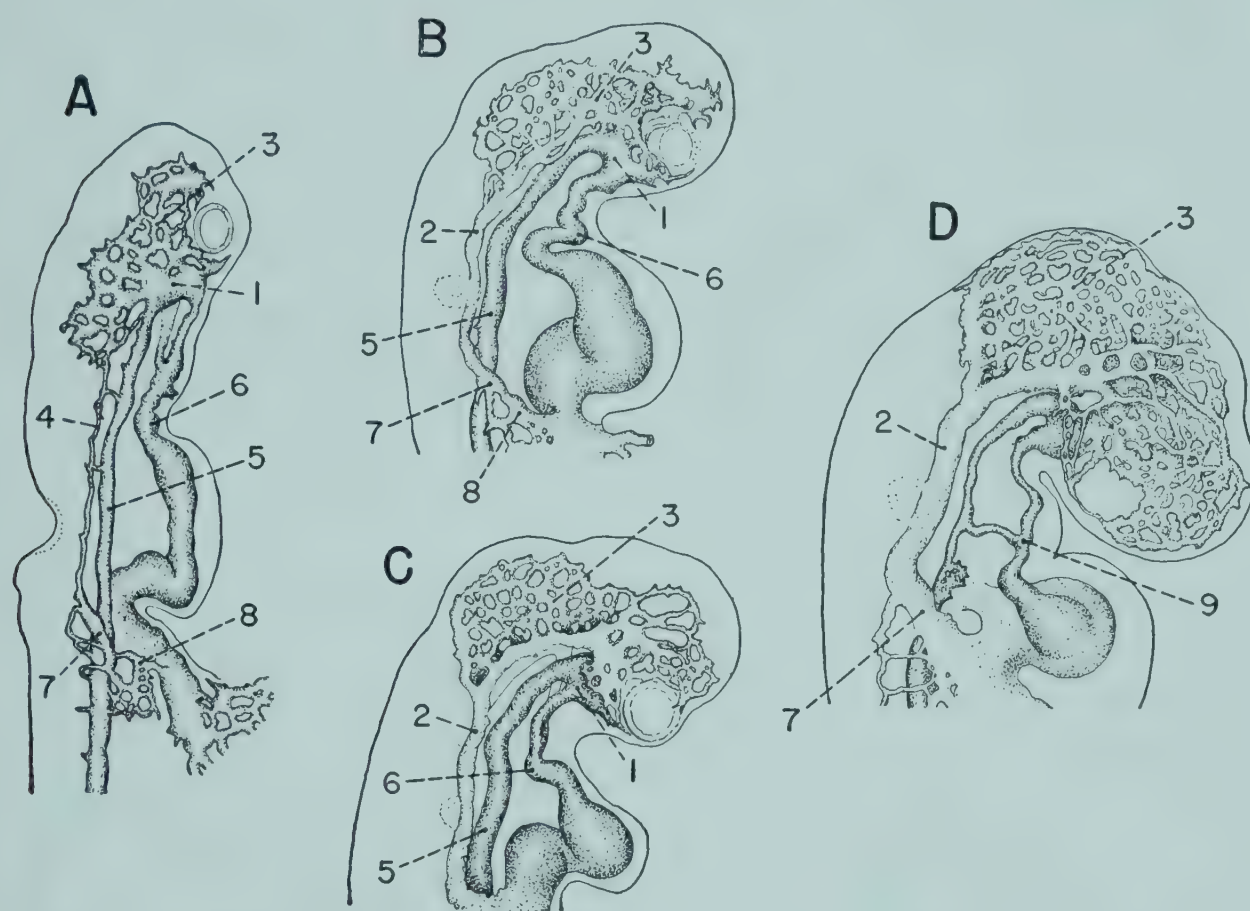
Within the same period, a deeply situated vascular plexus appears along the lateral surface of the midbrain and forebrain and the ventral surface of the latter (Fig. 246-A). This plexus is connected with the aorta in the forebrain and midbrain region and with the primitive vena capitis medialis in the hindbrain region. From this deep plexus there arises a superficial plexus opposite the forebrain and midbrain. As soon as the blood begins to circulate, at the 16-somite stage (Fig. 246-B), a venous channel is resolved from part of the superficial plexus at the level of the midbrain (Sabin, 1917b); this channel represents the most anterior part of the primitive cerebral vein.

The plexus continues to extend itself over the forebrain and midbrain, and small channels appear around the optic vesicle (Fig. 246-C and D). The small vessels around the eye are drained into the primitive cerebral vein by a larger vessel known at first as the inferior ophthalmic vein, although, in actuality, it is the primitive maxillary vein (Sabin, 1917b).

By the time the 29-somite stage is reached, a slender vessel has formed lateral to the vena capitis medialis through the junction of an outgrowth from the primitive maxillary vein with an outgrowth from the anterior cardinal vein (Stracker, 1916), as shown in Fig. 247-A. This new vessel, known as the vena capitis lateralis, thus forms an anastomosis between the anterior cardinal vein and the anterior cerebral vein; and it is also connected with plexuses in the maxillary, mandibular, and hyoid arches (Sabin, 1917b). The vena capitis lateralis is lateral to all the cranial ganglia except that of the trigeminal nerve. The appearance of this vein transforms the primitive cerebral vein into a vessel extending from the forebrain to the duct of Cuvier and consisting of three continuous portions: the anterior cerebral vein, the vena capitis lateralis, and the anterior



cardinal vein (the caudal portion of which becomes the jugular vein when the duct of Cuvier migrates to a more posterior position). The entire primary cerebral vein soon straightens out, and, on the fourth and fifth days, the transition points between its three segments are indistinguishable. The portion of the primary cerebral vein extending from the level of the trigeminal nerve to the level of the vagus nerve was called the "primary Stammvene" by Gelderen (1924a).



**Fig. 246.** Early development of the vessels of the head of the chick embryo. (Redrawn with rearrangements after Evans, 1909b.)

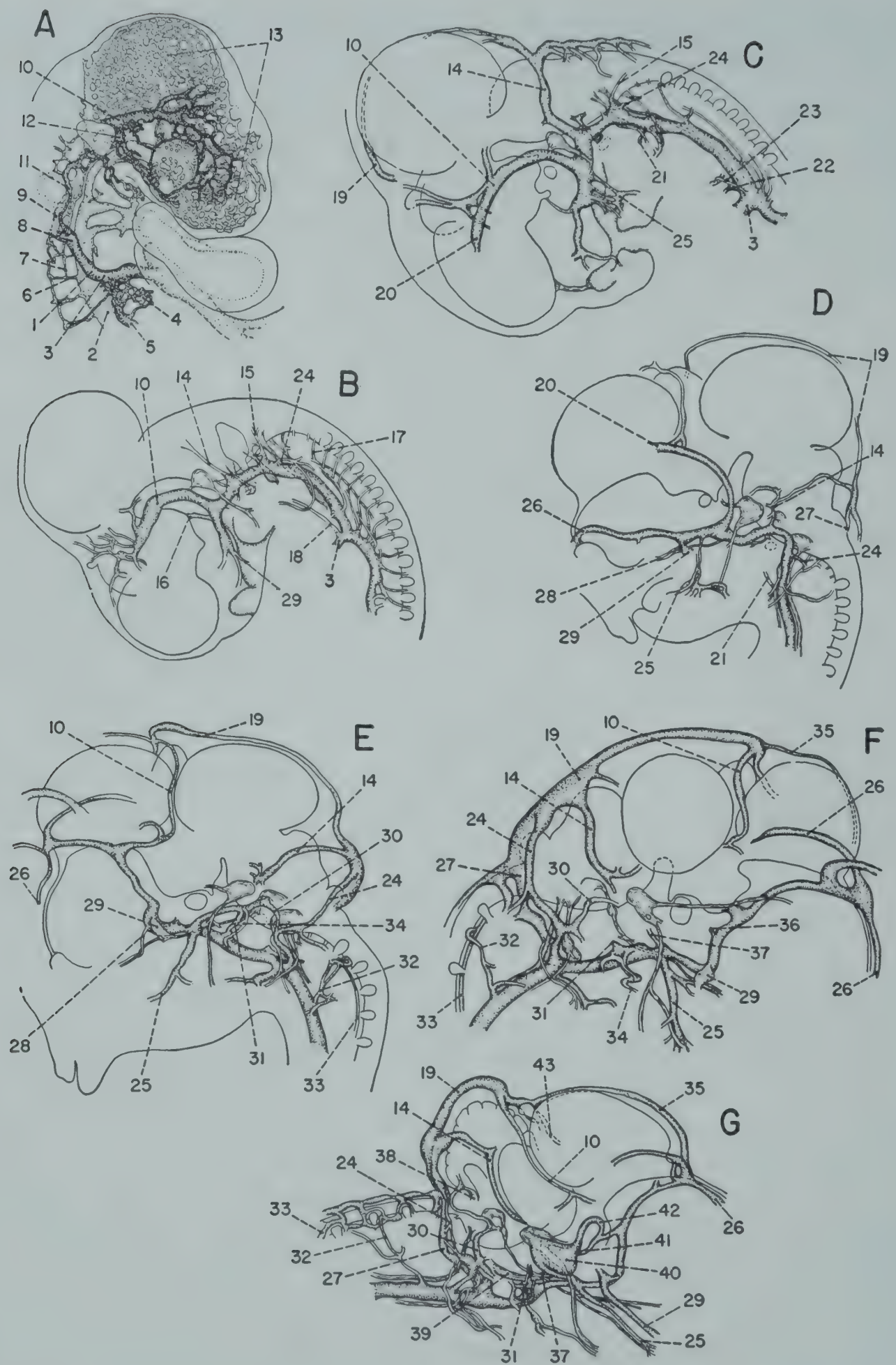
A, at 15 somites, the primary capillary plexus of the head, originating from the convexity of the first aortic arch, is drained by a capillary chain emptying into the main vitelline vein near its junction with the heart; B, at 17 somites, the primary capillary plexus of the head has extended. Its superficial portions are destined to form the main trunk of the vein traversing that region. C, at 20 somites, the vena capitis medialis, continuous with the anterior cardinal vein, has developed from the primary capillary plexus of the head; D, at 25 somites, tributaries of the anterior cardinal vein have differentiated, and the forebrain and midbrain muscles are completely invested by the plexus.

1, first aortic arch; 2, vena capitis medialis; 3, primary capillary plexus of the head; 4, capillary chain draining the primary head capillary plexus; 5, dorsal aorta; 6, ventral aorta; 7, anterior cardinal vein; 8, duct of Cuvier; 9, second aortic arch.

The vena capitis lateralis receives the blood from the forebrain and midbrain that formerly drained through the primitive vena capitis medialis. The latter very soon breaks up into a capillary plexus covering the entire hindbrain and becomes part of the system of vessels (both venous and arterial) of the pia mater (Sabin, 1917b).

The primary cerebral vein itself is a transitory vessel; its major portion





**Fig. 247.** Development of the veins in the head and neck of the chick embryo. (Redrawn with modifications after Hughes, 1934.)

A, at 2 days' incubation ( $\times 6$ ); B, at 5.5 days ( $\times 6$ ); C, at 6.5 days ( $\times 6$ ); D, at 7.5 days ( $\times 6$ ); E, at 8.5 days ( $\times 5$ ); F, at 12 days ( $\times 4$ ); G, at 21 days ( $\times 2$ ).

1, anterior cardinal vein; 2, dorsal aorta; 3, duct of Cuvier; 4, capillary plexus of umbilical vein; 5, posterior cardinal vein; 6, artery of third interspace; 7, basilar artery; 8, transverse vein of first interspace; 9, primitive vena capitis medialis; 10, anterior cerebral vein; 11, vena capitis lateralis; 12, primitive maxillary vein; 13, capillary plexus; 14, middle cerebral vein; 15, posterior cerebral vein; 16, hypophysis; 17, sixth intersegmental vein;



is destined to atrophy. Its replacement by other vessels has been best described by Gelderen (1924a) and Hughes (1934). The main steps in the process, as determined by Gelderen, are diagrammed in Fig. 248. Inspection of this figure reveals that new venous channels form both dorsal and ventral to the primary vein and that the chief dorsal vein, the middorsal longitudinal sinus, is the principal permanent vessel draining the brain.

The middorsal (sagittal) sinus arises through the coalescence in the midline of a superficial plexus. Over the forebrain and the anterior surface of the optic lobes, this plexus is connected with the anterior cerebral vein (Fig. 247-B), which lies on the lateral surface of the diencephalon (thalamus). Over the posterior surface of the optic lobes, and backward, the plexus is connected with a new vessel, the middle cerebral vein (cf. Fig. 247-B). This vein may be seen at the 32-somite stage growing dorsalward over the hindbrain as a branch given off from the lateral cerebral vein just anterior to the otic vesicle. (A transitory vein, the posterior cerebral vein, may also be seen in Fig. 248-B posterior to the otic vesicle.) The middorsal sinus begins to form in the region of the optic lobes at the end of the third day (*Evans, 1909b*). At the 6.5-day stage (cf. Fig. 247-C), the sinus exists in the form of discontinuous anterior and posterior segments. At the 8.5-day stage (cf. Fig. 247-E; Fig. 248-F), its two segments unite over the optic lobes (*Hughes, 1934*).

After 8.5 to 10 days of incubation, the proximal portions of both the anterior and the middle cerebral veins atrophy, and these veins thus lose their connections with the primary head vein (Fig. 247-E; Fig. 248-F and G). The portion of the anterior cerebral vein that disappears at this time represents the greater part of the vessel of the 29-somite stage.

The ventral vessel that is destined to replace most of the posterior part of the primary head vein was called the "secondary Stammvene" by Gelderen (1924a). This may be seen after about 6.5 or 7.5 days of incubation (cf. Fig. 247-C and D) growing out from the head vein just posterior to the twelfth cranial nerve (*Hughes, 1934*). It extends itself cephalad and, after 8.5 days' incubation, rejoins the primary cerebral vein at the level

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18, secondary subclavian vein; 19, middorsal sinus; 20, ophthalmic continuation of the anterior cerebral vein; 21, posterior root of the secondary Stammvene; 22, definitive secondary subclavian artery; 23, superseded connection of secondary subclavian artery and duct of Cuvier; 24, external occipital vein; 25, maxillary vein; 26, ethmoid vein; 27, occipital vein; 28, palatine vein; 29, vein of the chorioid fissure; 30, primary Stammvene; 31, pretympanic connection between primary and secondary Stammvene; 32, lateral occipital vein; 33, vertebral vein; 34, external facial vein; 35, anterior continuation of the middorsal sinus; 36, root of the anterior cerebral vein; 37, anterior root of the primary Stammvene; 38, floccular vein; 39, carotid vein; 40, temporal rete; 41, vein to the sella turcica; 42, vein to the temporal rete; 43, vein to the anterior chorioid plexus.



where the maxillary vein is given off (Fig. 247-E; Fig. 248-F). The bypassed portion of the primary vein subsequently disappears (Fig. 248-G), leaving an anterior and a posterior stump. The anterior stump may be seen in the 12-day embryo (cf. Fig. 247-F); it extends dorsally from the secondary Stammvene, opposite the root of the maxillary vein, and runs to the temporal rete. Between the twelfth day and the hatching date (Fig. 247-G), the posterior stump acquires a connection with the floccular vein (from the flocculus of the cerebellum) and is continued into the vena auris interna; it is also joined by the carotid vein (Hughes, 1934).

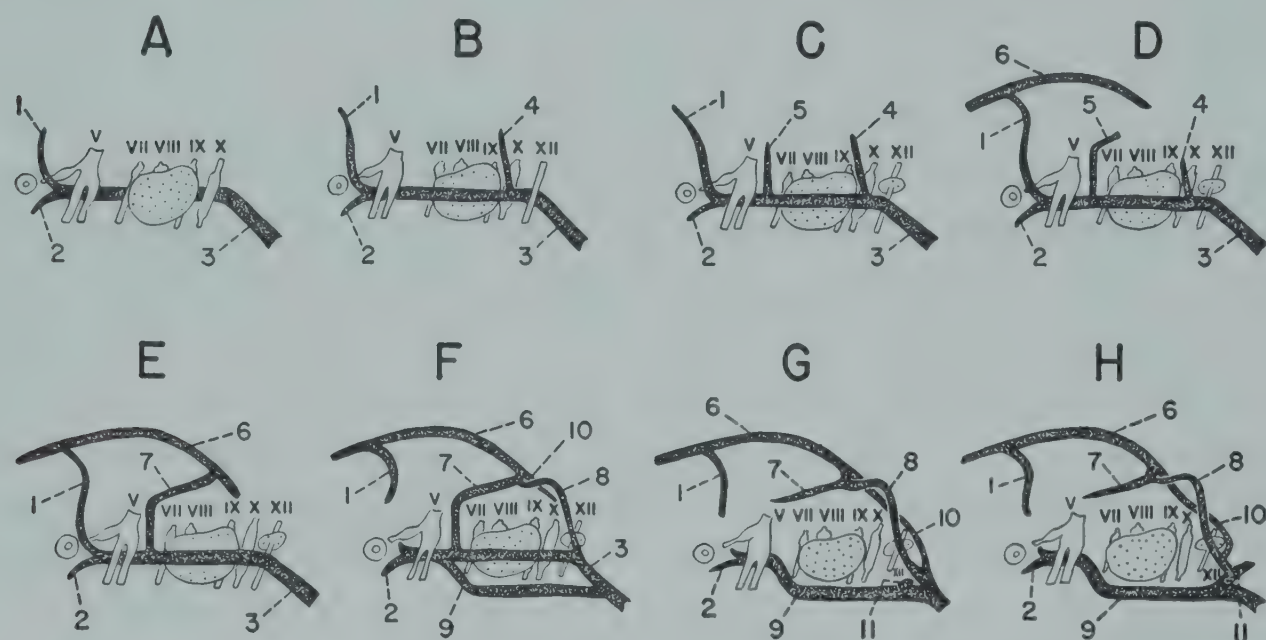


Fig. 248. A schema showing eight stages in the development of the dural veins during development of the embryo of the chick pictured in relation to the eye (concentric circles), otic vesicle (stippled oval), and cranial nerves (Roman numerals). (Redrawn with modifications after Gelderen, 1924a.) Incubation ages based on Keibel's plates (Keibel and Abraham, 1900).

A, at less than 100 hours of incubation; B, at 104 hours; C, at 114.5 hours; D, at 172 hours; E, at 193.5 hours; F, at 8.5 days; G, at 10 days; H, at 12 days.

1, anterior cerebral vein; 2, infraocular vein; 3, anterior cardinal vein; 4, posterior cerebral vein; 5, middle cerebral vein; 6, superior longitudinal venous sinus; 7, anastomotic vein; 8, external occipital vein; 9, secondary Stammvene destined to become the intracranial portion of the internal jugular vein; 10, occipital vein; 11, occipital anastomotic vein. Roman numerals indicate the cranial nerves.

A cross-anastomosis forms between the two secondary Stammvenen after the 8.5-day stage. From this cross-anastomosis, venules grow dorsally in the midline and bifurcate around the hindbrain to meet similar bifurcations of the middorsal longitudinal sinus which are growing downward. After the tenth or twelfth day (cf. Fig. 247-F; Fig. 248-G), the venules develop into the paired occipital veins, one on either side of the midline (Gelderen, 1924a; Hughes, 1934). At the same time, each occipital vein acquires a connection with the external occipital vein. The latter is formed at the 8.5-day stage (cf. Fig. 247-E; Fig. 248-F) by the junction of its intracranial dorsal root, growing down from the middorsal sinus, with its extra-



cranial ventral root, a branch of the "primary Stammvene" first visible at 4.5 days. According to Gelderen (1924a), the ventral portions of the external occipital veins disappear, but Hughes (1934) found them present at hatching (cf. Fig. 247-G).

In the lapwing (*Vanellus vanellus*), the head veins develop in essentially the same way as in the chick. The external occipital vein does not form in the duck (*Anas platyrhynchos*), according to Gelderen (1924b).

The fate of the large primitive veins lying in the region of the forebrain and diencephalon has been described by Hughes (1934). The "primitive maxillary vein" of Sabin (1917b) is regarded by Hughes as the "anterior continuation of the head vein," from which the definitive maxillary vein is later (by the 6.5-day stage) given off; the anterior cerebral vein is considered as the chief tributary of this anterior trunk of the head vein. By the middle of the sixth day, the capillary plexus over the orbit condenses into the supraorbital vein, which constitutes a branch of the anterior cerebral vein. The supraorbital vein grows forward outside the cranial wall until, at the 8.5-day stage, it joins the anterior trunk of the head vein in the olfactory region. By this time, however, the proximal portion of the anterior cerebral vein—including the portion that gives off the supraorbital vein—has atrophied, and the supraorbital vein has therefore become a tributary of the anterior trunk of the head vein. The latter has meantime elongated even farther anteriorly, for, at about the 7-day stage, it grows out with the olfactory nerve and ethmoidal artery as the ethmoidal vein (cf. Fig. 247-D).

The head veins of the chick at hatching are pictured in Fig. 247-G. Comparison with Fig. 243-G reveals the rather close correspondence between the paths of arteries and veins.

### *The Intraencephalic Vessels*

Capillaries begin to penetrate the substance of the chick embryo's brain at some time between the forty-eighth (Williams, 1937) and the eighty-fourth (Feeney and Watterson, 1946) hour of incubation. At the latter time, blood vessels are present in all parts of the brain except the telencephalon. In the diencephalon they are found laterally, but in the mesencephalon, metencephalon, and myelencephalon they enter ventrally as paired vessels arising from the basilar artery (Feeney and Watterson, 1946). The vessels do not show a segmental or neuromeric arrangement, one intersomitic interval being the equivalent of the distance between about eight vessels (Hughes, 1934). In the three posterior brain vesicles, the dorsal tips of the penetrating capillaries have sprouted cephalad and caudad, and the sprouts are anastomosing to form bilateral endoneural sinuses (Fig. 249-B); these sinuses become continuous with those of the 4-day embryo's spinal cord.



At the 4-day stage, a plexus is forming on the surface of the ependymal layer in the regions penetrated at 3.5 days, and in the telencephalon as well. Capillaries have now penetrated this brain vesicle ventrolaterally, in the corpora striata. Connections across the ventral midline are present only in the diencephalon and the mesencephalon; in the latter, lateral vessels are now more numerous than ventral ones.

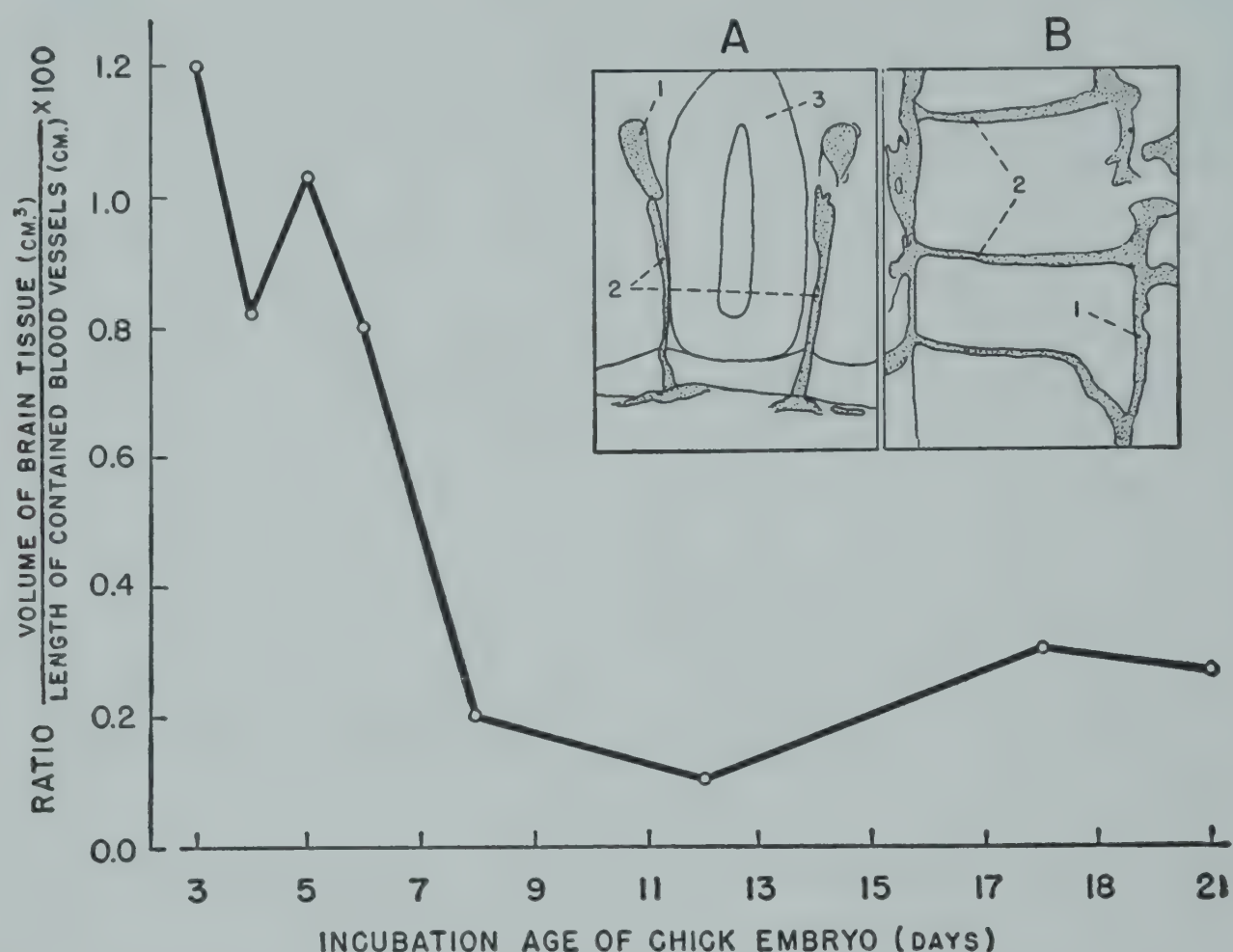


Fig. 249. The development of the intraneural vascular pattern in the embryo of the chick.

Graph showing the ratios of volume of hindbrain tissue to the length of contained blood vessels at various stages in the development of the embryo of the chick from 3 days' incubation to hatching. (Redrawn with modifications after Williams, 1937; *Inserts* after Feeney and Watterson, 1946.)

*Inserts:* A, a transverse section of the spinal cord at the level of the cervical enlargement of a chick embryo at 4 days of incubation ( $\times 150$ ); B, a longitudinal section of the mesencephalon of a chick embryo at 4 days of incubation ( $\times 35$ ).

1, endoneural sinus; 2, penetrating capillary; 3, ependymal layer.

At 5 days, it can be seen that the vascularity of the basal plate is greater than that of the alar plate in all parts of the brain. Ventral penetrating vessels predominate in the myelencephalon only. Elsewhere, lateral penetrating vessels continue to increase in number and to form interconnections lateral to the ependyma. Vessels have penetrated the entire ventral surface of the optic lobes and have formed endoneural sinuses just within the ependymal zones; other vessels enter laterally.

In the 6-day embryo, capillaries enter the alar plates of the myelencephalon, the dorsal part of the thalamus, and the ventrolateral walls of



the infundibulum. The plexiform interconnections between them are extending peripherally. Connections are present across the ventral midline in all regions except the myelencephalon and the telencephalon.

At the 7-day stage, the penetration of capillaries has extended dorsally, especially in the metencephalon and mesencephalon. Vessels now enter the cerebral hemispheres dorsomedially and ventromedially, having extended from the dorsal and ventrolateral regions. The peripheral extension of interconnections between penetrating vessels is particularly marked in the myelencephalon.

The further development of the intraencephalic vessels occurs in conjunction with the development of the special structures of the various brain regions. Thus, in the cerebellum, most of the capillary penetrations occur through the invaginations that demarcate the primary and secondary folia. By the sixteenth day, an extensive vascular plexus is present in the mantle zone of each convolution and is connected to the outside by vessels that pass through the cortex without branching. In the mesencephalon, changes are associated with the development of the strata of the optic tectum. Here the interconnections do not spread from ependyma to periphery, as in the rest of the brain; vessels are at all stages most numerous approximately midway between the cavity of the optic lobe and the periphery, in the zone containing the largest nerve cell bodies; from this zone, they spread in both directions. Interconnections between penetrating capillaries do not appear in proximity to the endoneural plexus until the tenth day. They first form in the outermost zone on the fourteenth day. Until this time, the penetrating vessels of the optic lobes are strikingly radial, paralleling the nervous elements, and the interconnections between them are almost at right angles. The later changes in the diencephalon are largely confined to the optic chiasma, to which vessels extend on the eighth day from the plexus within the diencephalon and on the ninth day from the perineural plexus. They enter the optic nerves dorsally, run parallel to the nerve fibers, and form few interconnections with the vessels entering the chiasma with the opposite nerve.

From the third to the twenty-first incubation days, the volume of the chick's brain tissue increases at a relatively slower rate than the length of the intraencephalic blood vessels, except on the fifth and the eighteenth days (*Williams, 1937*). This relationship between growth rates may be inferred from Fig. 249, which shows graphically the changing ratios between tissue volume and blood vessel length in the hindbrain throughout incubation.

### The Vascularization of the Extremities

The vascularization of the limb buds is preceded by the formation of capillary plexuses. Arterial channels are connected with the dorsal aorta by



multiple vessels which are gradually replaced by a principal artery. The first venous vessels to arise are in a marginal position.

### *The Vessels of the Wing*

In the adult bird, blood is brought to the wing through the brachial artery, one of the two main branches of the subclavian artery. In the chick embryo, the brachial artery is not recognizable until the close of the fourth incubation day, when the wing bud begins to grow out; the subclavian artery supplies the early wing bud directly.

The development of the definitive subclavian artery is characterized by the establishment of a primary vessel and its replacement by a secondary

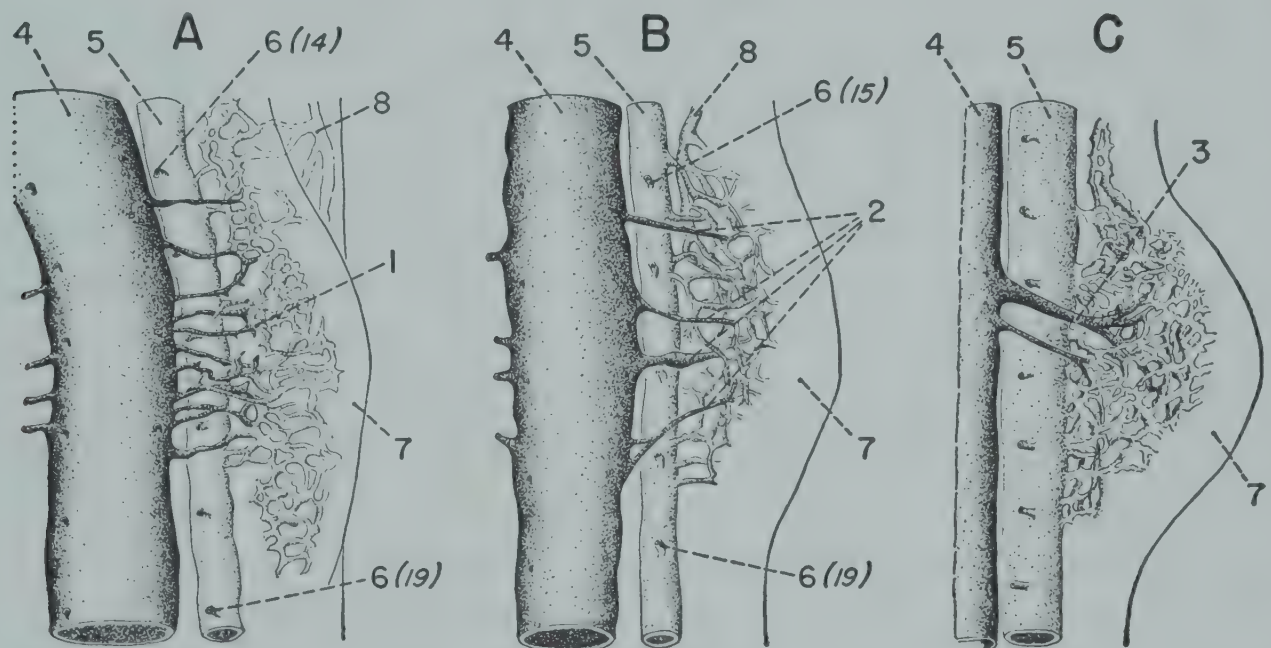


Fig. 250. Differentiation of the primitive subclavian artery in the developing chick, shown on the right side only. (Redrawn with modifications after Evans, 1909a.)

A, the primary subclavian capillary plexus in the region of the developing wing bud; B, the stage of multiple subclavian arteries, which replace the capillary plexus; C, the stage of one primary subclavian vessel, which becomes the primitive subclavian artery, later replaced by the definitive vessel. All  $\times 25$ .

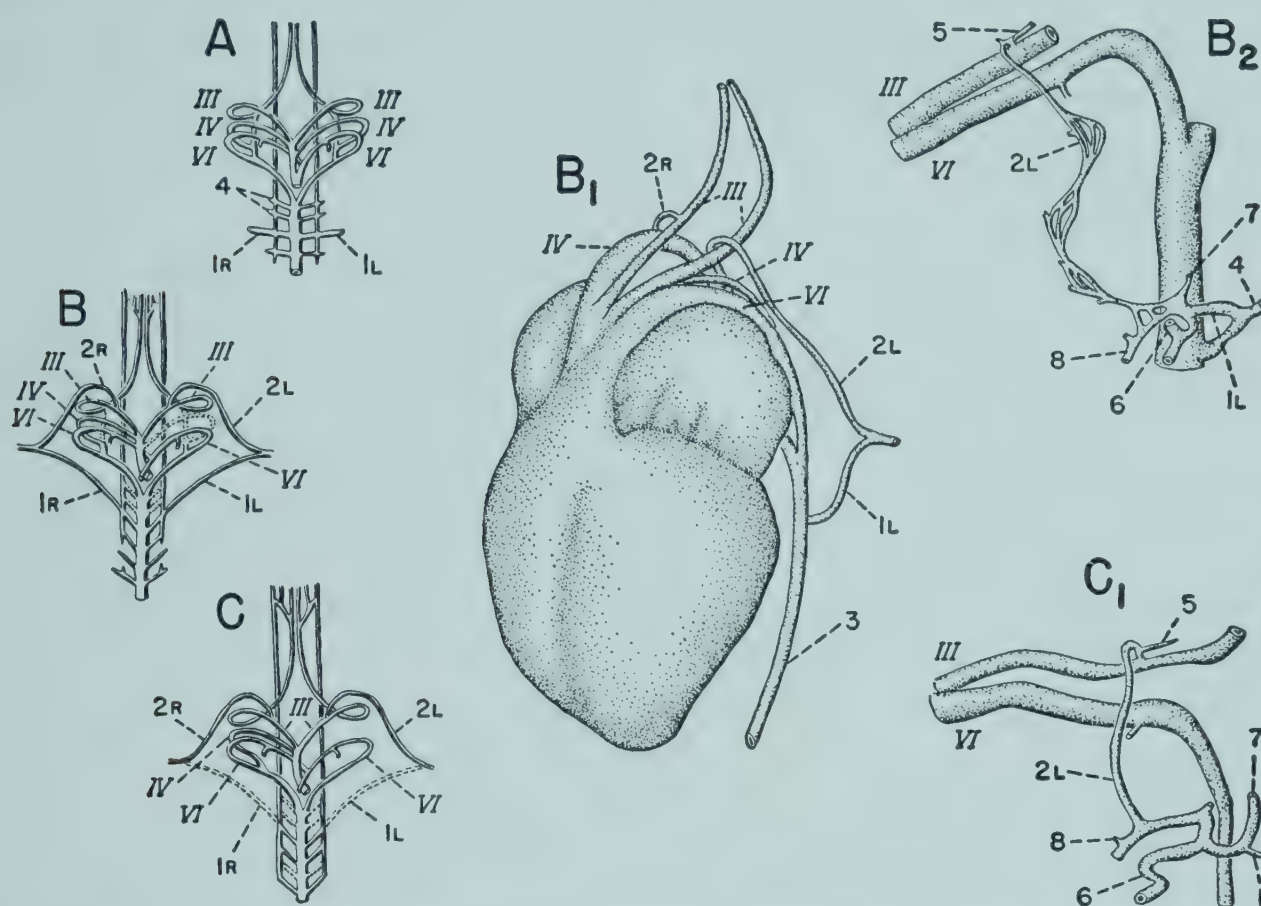
1, primary subclavian plexus; 2, multiple segmental subclavian vessels; 3, primary subclavian artery; 4, aorta; 5, posterior cardinal vein; 6, dorsal intersegmental vein, number indicated in parenthesis; 7, wing bud; 8, umbilical vein.

vessel. The primary artery is a direct branch of the dorsal aorta; the secondary artery arises from the third aortic arch and is eventually brought to the wing level by the backward migration of the aortic arches.

A capillary plexus originates in the wing bud as a downgrowth from the plexus lying in the somatopleure in the angle between the duct of Cuvier and the posterior cardinal vein (cf. Fig. 257-A, B, and C). Most of the latter plexus condenses into the umbilical vein; but, as the capillaries reach the level of the very early wing bud, they are met by, and anastomose with, capillaries growing out from the lateral wall of the aorta (cf. Fig. 254-A; Fig. 257-D). In 60- to 65-hour (about 32-somite) chick embryos, six to ten capillaries lead off from the aorta (Fig. 250-A) at the



level of the fourteenth to twentieth somites and anastomose with the capillaries of the wing bud (*Evans, 1909a*). Within 12 hours (*Evans, 1909a*), these capillaries, which are not segmental in arrangement (*Bakst and Chafee, 1928*), atrophy and give way to fewer and larger vessels (cf. Fig. 250-B), arranged metamerically. The same condition has been observed in the penguin, *Eudyptes* sp. (*Müller, 1907*). *Evans (1909a)*



**Fig. 251.** Replacement of the primary subclavian artery by the definitive secondary vessel in the chick embryo. (Redrawn with modifications *B<sub>1</sub>*, after *Sabin, 1905*; *A, B, C*, after *Krassnig, 1913b*; *B<sub>2</sub>, C<sub>1</sub>*, after *Bakst and Chafee, 1928*.)

The diagrams on the left represent the three main stages in replacement: *A*, the existence of only a primary subclavian artery; *B*, the coexistence of primary and secondary subclavian arteries, both functional; *B<sub>1</sub>*, the coexisting primitive and definitive subclavian vessels during the sixth day of incubation, shown in relation to the heart ( $\times 12$ ); *B<sub>2</sub>*, left lateral view of the same vessels ( $\times 15$ ); *C*, the persistence of the secondary subclavian vessels coupled with the atrophy and disappearance of the primary subclavian arteries; *C<sub>1</sub>*, left lateral view of the definitive subclavian artery of a chick incubated 6 days 17 hours ( $\times 15$ ).

1L, 1R, left and right primary subclavian arteries; 2L, 2R, left and right definitive subclavian arteries; 3, dorsal aorta; 4, dorsal segmental artery; 5, ventral aorta; 6, ventral wing vessel; 7, dorsal wing vessel; 8, internal mammary artery; I-VI, aortic arches.

observed four such vessels in the 34-somite chick embryo and five to six in a 38-somite duck (*Anas platyrhynchos*) embryo; *Rabl (1907a)* reported three in the duck embryo. The multiple primary subclavian arteries arise at the levels of the sixteenth to nineteenth intersomitic spaces (*Evans, 1909a*). They in turn disappear (cf. Fig. 250-C), and, by the time the 45-somite stage is reached, only one primary subclavian artery usually remains (Fig. 251-A); this is generally considered to originate in the region of the



eighteenth interspace (*Hochstetter*, 1890*b*, 1892; *Sabin*, 1905; *Evans*, 1909*a*; *Hughes*, 1934), although the fourteenth (*Krassnig*, 1913*b*) and the sixteenth (*Fleming*, 1926) have also been designated. *Sabin* (1905) noted that the primary subclavian artery of the 80-hour chick is not identical with the eighteenth segmental artery; the latter springs from the dorsal side of the aorta, whereas the primary subclavian arises laterodorsally. The two vessels come to have a common root after 5 days' incubation, however.

In the meantime, the secondary subclavian artery has formed. *Mackay* (1888) described it in the 4-day chick embryo and the 5-day duck (*Anas platyrhynchos*) embryo as running from the ventral end of the third aortic arch into the duct of Cuvier (cf. Fig. 242-A). *Hochstetter* (1890*b*) and *Sabin* (1905) considered this vessel as a vein draining the base of the hyoid arch and asserted that the definitive subclavian does not arise until the chick's sixth day of incubation. *Hughes* (1934), however, regarded the "vein" and the secondary subclavian artery as identical because they obviously are derived from the same capillary system. This author stated that the vessel arises from the duct of Cuvier on the third incubation day and grows forward, joining the capillary plexus of the hyoid arch on the fourth day; *Bakst and Chafee* (1928) considered it as a cranially directed branch of the primary subclavian artery, first apparent on the fourth day. At the 5-day stage, it is connected with the ventral aorta (*Bakst and Chafee*, 1928) immediately anterior to the junction of the latter with the third aortic arch, the hyoid arch having meantime disappeared (*Hughes*, 1934). A day later, the secondary subclavian artery is found lateral to the duct of Cuvier, to which it is no longer connected. It then joins the primary subclavian (Fig. 251-B,  $B_1$ , and  $B_2$ ) in the tissue between the base of the wing and the lung (*Sabin*, 1905). The primary vessel now starts to atrophy (*Bakst and Chafee*, 1928); but both arteries jointly supply the wing bud until the beginning of the eighth day, when the primary subclavian rapidly disappears (Fig. 251-C and  $C_1$ ). The heart is now posterior to the wing bud, and the secondary subclavian artery is therefore relatively much shorter and is directed laterally (*Sabin*, 1905). The ventral aorta has disappeared and the definitive subclavian artery is given off directly from the third aortic arch (*Bakst and Chafee*, 1928).

The definitive subclavian artery is established in an identical manner in the chick (*Mackay*, 1888; *Hochstetter*, 1890*b*; *Sabin*, 1905; *Bakst and Chafee*, 1928; *Hughes*, 1934); the duck, *Anas platyrhynchos* (*Mackay*, 1888; *Rabl*, 1906, 1907*a*); a pheasant, the gull, *Larus* sp. (*Bakst and Chafee*, 1928); and the ostrich, *Struthio camelus* (*Fleming*, 1926); see Fig. 252. Its origin explains why this vessel, in birds, lies ventral to the superior vena cava and the vagus nerve, rather than dorsal thereto, as in mammals. The mammalian vessel arises from the dorsal aspect of the fourth aortic arch.

Development of the more distal arteries of the wing begins before the de-



definitive subclavian artery has completely replaced the primitive vessel. By the 4.5-day stage (Fig. 253-A), the proximal portion of the lengthening wing bud can be designated as the upper wing, into which the subclavian artery extends axially as the brachial artery (*Hochstetter, 1891*). In the penguin (*Eudyptes* sp.) embryo, there are three arteries rather than one in this region (*Müller, 1907*). Distally, the brachial artery continues into a luxuriant capillary network from which the interosseous artery is differentiating as an irregular channel on the dorsal side of the wing bud. Before the end of the chick's fifth incubation day, the ulnar artery begins to condense from

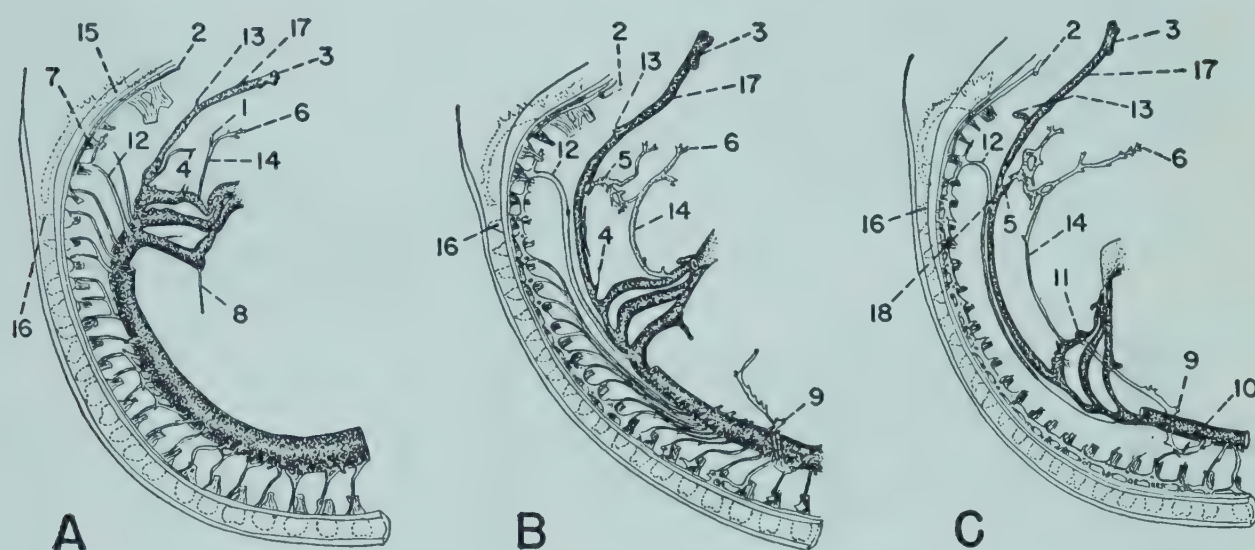


Fig. 252. Successive stages in the development of the vertebral, subclavian, and external carotid arteries of the ostrich, *Struthio camelus*. (Redrawn with modifications after Fleming, 1926.)

A, in the 12-mm. ostrich embryo; B, in the 16-mm. ostrich embryo; C, in the 18-mm. ostrich embryo.

1, primary inferior alveolar artery; 2, basilar artery; 3, cephalic sinus; 4, primary dorsal external carotid artery; 5, secondary dorsal external carotid artery; 6, lingual artery; 7, third root of hypoglossal nerve; 8, pulmonary artery; 9, subclavian artery; 10, primary subclavian artery; 11, secondary subclavian artery; 12, first spinal dorsal segmental artery (vertebral artery); 13, stapedial artery; 14, ventral external carotid artery; 15, vagus nerve; 16, third spinal nerve; 17, dorsal carotid artery; 18, connection of vertebral artery with dorsal carotid artery.

the capillary network on the ventral and posterior side of the "forearm." By the 6.5-day stage (Fig. 253-B), the ulnar artery closely parallels the interosseous artery, from which it branches just above the elbow; like the interosseous artery, the ulnar artery ends distally in the small, still irregular arteries of the digital region. At the 8-day stage, the ulnar artery has enlarged at the expense of the interosseous artery, which has lost some of its connections with the digital arteries. The latter are now clearly differentiated. The radial artery and the artery of the ulnar nerve branch from the interosseous artery at the elbow and run longitudinally in the forearm, along the anterior and posterior borders. In the 10-day embryo (Fig. 253-C), these two arteries lie lateral to the ulna and radius, respectively,



while the ulnar and interosseous arteries course between the bones (Swienty, 1937).

The first venous channel to drain the anterior extremity is the umbilical vein, which, as already mentioned, is in communication with the early capillary network of the wing bud (Evans, 1909b). In the adult bird, however, blood from the wing passes through the subclavian vein to the superior vena cava. The subclavian vein has appeared in the 4.5-day chick embryo as a branch of the posterior cardinal vein. At the base of the wing bud it

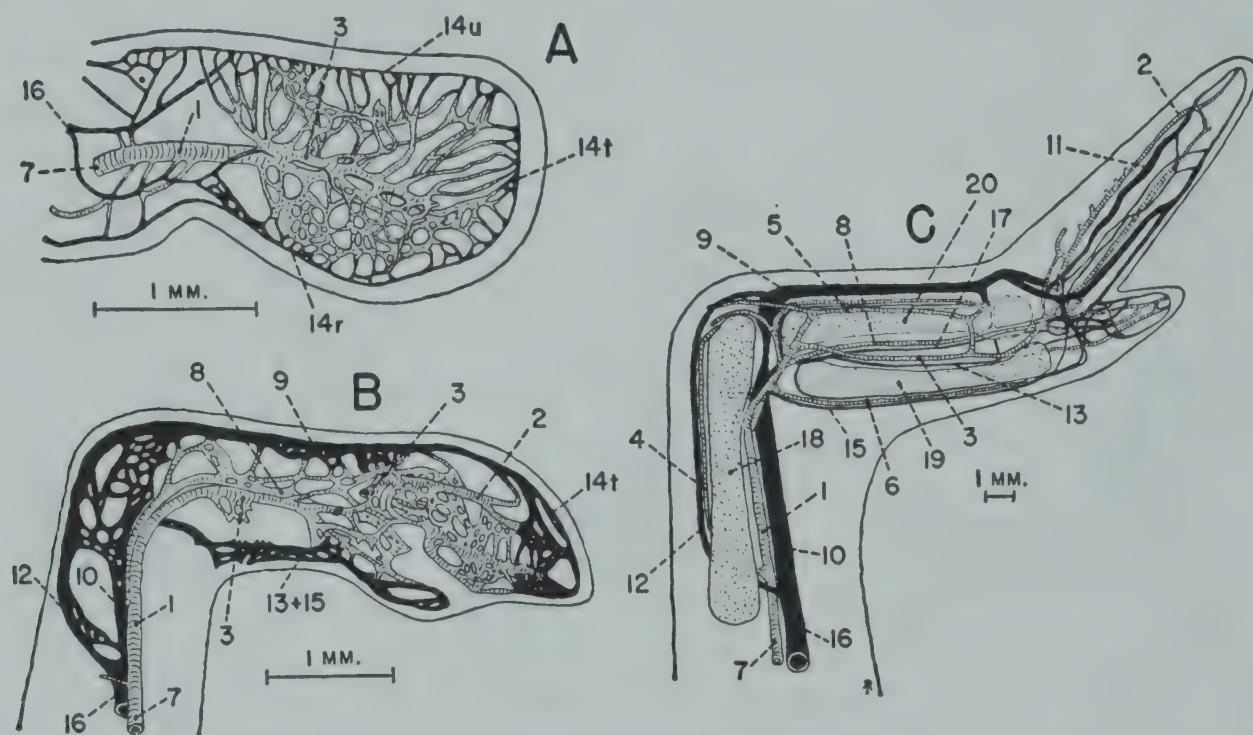


Fig. 253. Successive stages of vessel development in the wing of the chick embryo. (Redrawn with modifications after Swienty, 1937.)

A, at 108 hours, the brachial artery and the marginal vein are the most completely developed vessels; B, at 158 hours (6 days 12 hours), the ulnar, interosseous, and digital arteries have formed, and blood is drained from the wing bud by the humeral vein and the differentiating radial and interosseous veins, which have partially replaced the marginal vein; C, at 242 hours (10 days 2 hours), all the main vessels have fully differentiated, and the adult vascular pattern is determined. All in scale.

1, brachial artery; 2, digital artery; 3, interosseous artery; 4, humeral artery; 5, artery of the ulnar nerve; 6, radial artery; 7, subclavian artery; 8, ulnar artery; 9, basilic vein; 10, brachial vein; 11, digital vein; 12, humeral vein; 13, interosseous vein; 14r, radial portion of the marginal vein; 14t, terminal portion of the marginal vein; 14u, ulnar portion of the marginal vein; 15, radial vein; 16, subclavian vein; 17, ulnar vein; 18, humerus; 19, radius; 20, ulna.

divides into two branches which are continuous with the irregular marginal vein (cf. Fig. 253-A) bordering the wing bud. The marginal vein, which unites the dorsal and ventral capillary networks of the wing bud (Swienty, 1937), is arbitrarily divided into three sections: the radial marginal vein anteriorly, the ulnar marginal vein posteriorly, and the terminal marginal vein distally (connecting the two other sections). The ulnar marginal vein is the first to lose its plexiform character and, by the 6.5-day stage (cf. Fig. 253-B), is clearly the basilic vein of the adult (Hochstetter, 1891). The



basilic vein now continues at the elbow into a plexus from which, in the upper wing, the brachial vein is condensing axially and the humeral vein posteriorly, near the surface. These veins join more proximally to form the subclavian vein. Along the anterior surface of the "forearm," the radial and interosseal veins are beginning to form from a plexus. The original radial marginal vein has disappeared, except, perhaps, in the terminal portion of the "thumb." In the 8-day embryo, the radial vein is superficial to the radial artery, and the interosseal vein lies medial to it. Both branch from a new vein, the ulnar vein, which accompanies the ulnar artery and springs from the brachial vein. The brachial vein is now much larger than the humeral vein. At the 10-day stage (cf. Fig. 253-C), the humeral vein is relatively even smaller. The brachial vein continues directly into the basilic vein, which in turn continues distally into the digital veins. The latter first become clearly evident in the 8-day embryo and are apparently derived from the original radial terminal vein (Swienty, 1937).

### *The Vessels of the Leg*

The primary circulation of the leg is established in exactly the same way as that of the wing. In the 32-somite (2.5-day) chick embryo, several dorsal segmental vessels (lying at, and posterior to, the twenty-seventh interspace) anastomose with independent lateral offshoots from the aorta to form a capillary plexus in the limb bud (Fig. 254-B). This plexus lies dorsal to the plexus from which the caudal aorta arose and is caudal to the level reached at this time by the posterior cardinal vein. These capillaries eventually give rise to the chief arteries of the leg (Evans, 1909a), the crural artery and the more posterior ischiadic or sciatic artery, both of which arise directly from the aorta. The crural artery is seen in the 5-day chick embryo as a branch of the aorta (Miller, 1903). Hochstetter (1890a) recognized the segmental nature of the sciatic and crural arteries and stated that a different number of segments may intervene between them in different birds—one in the great Müller Amazonian parrot (*Amazona mülleri*), and two in the chicken, the European crested jay (*Garrulus glandarius*), the goatsucker (*Caprimulgus europaeus*), and the gray-headed Amazonian parrot (*Amazona* sp.). In the chicken, the crural artery is less important than the sciatic (Zuckerlandl, 1894); the reverse is true, however, in some birds, including the *Pipridae*, *Cotingidae*, *Musophagidae*, and *Spheniscidae*.

The development of the arteries distal to the chick's knee joint has been described by Zuckerlandl (1894). In the 80- to 90-hour chick, the sciatic artery divides into a dorsal and a plantar branch in the tarsal region. The dorsal branch (the tibial artery) passes outward between the anlagen of the bones in this region, but, as incubation proceeds, the place of emergence is shifted to a continually higher level, until, in the 7-day embryo, it lies



closer to the knee than to the ankle. Meantime (by the 6-day stage), the plantar branch of the sciatic artery has begun to atrophy. Interdigital arteries become visible in the 5-day embryo (*Hochstetter, 1891*).

In its first stages, the development of the leg veins resembles that of the wing veins. Thus, in the 80- to 90-hour chick embryo, there is a marginal vein, of which the anterior portion is designated the tibial marginal vein and the posterior portion the fibular marginal vein (*Hochstetter, 1891*). The primary drainage channels of the leg bud capillaries are the subintestinal veins, and the umbilical veins (*Grafe, 1906; Evans, 1909b*); cf. Fig. 255-D. In the 90-hour chick, the tibial marginal vein drains into the umbilical vein and the fibular marginal vein into the posterior cardinal

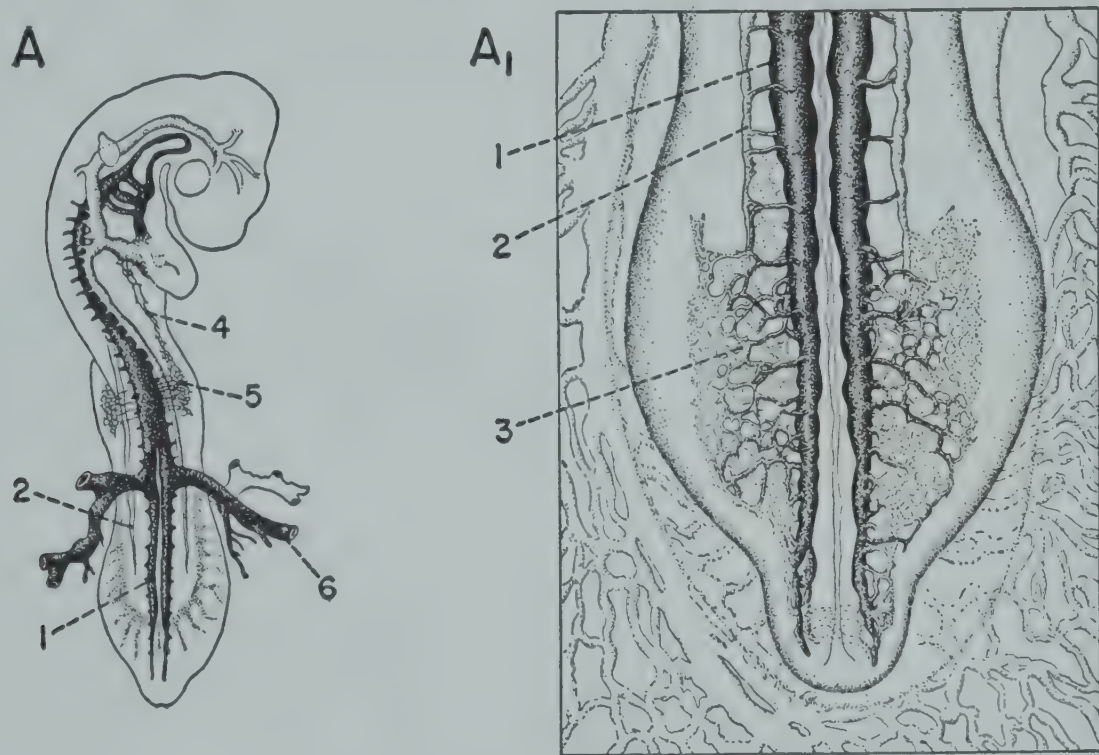


Fig. 254. The primary capillary plexus in the anterior and posterior limb buds of the 32-somite chick embryo, prior to the development of the sciatic arteries. A,  $\times 20$ ; A<sub>1</sub>,  $\times 60$ . (Redrawn with modifications after *Evans, 1909a*.)

1, left aorta; 2, left posterior cardinal vein; 3, capillary plexus of posterior limb bud; 4, right umbilical vein; 5, capillary plexus of anterior limb bud; 6, vitelline artery.

vein (*Hochstetter, 1891*); the subintestinal veins have already entered the retrogressive phase that culminates in their disappearance by the end of the fifth day (*Grafe, 1906*). At the 4.5-day stage, a superficial capillary network forms near the base of the leg bud, and a small secondary vein develops from this network in the 5-day embryo. This secondary vein, called the hypogastric vein by *Hochstetter (1888)*, will become the vessel known as the internal iliac or sciatic vein. It empties temporarily into the umbilical vein but very soon transfers its mouth to the posterior cardinal vein (*Hochstetter, 1891*). The internal iliac vein, after anastomosing first with the tibial marginal vein and then with the fibular marginal vein, takes over the circulation of these vessels, which consequently disappear on the sixth and the seventh day, respectively. The digital capillaries have meantime



made connections with the internal iliac vein. The latter has acquired a new, deep branch, springing from a point near its junction with the posterior cardinal vein and paralleling the axial artery of the leg. The deep (tertiary) vein, which reaches to the knee in the 6.5-day embryo, has grown as far as the ankle in the 7-day embryo and is joined there by numerous small veins. As the tertiary (femoral or external iliac) vein increases in size, the internal iliac vein becomes smaller; in the 8-day embryo, the latter appears as a small branch of the posterior cardinal vein, the caudal portion of which remains in the adult as the hypogastric vein (although the portion between the external and internal iliac veins is sometimes considered as a continuation of the internal iliac vein).

### The Pulmonary Veins and Arteries

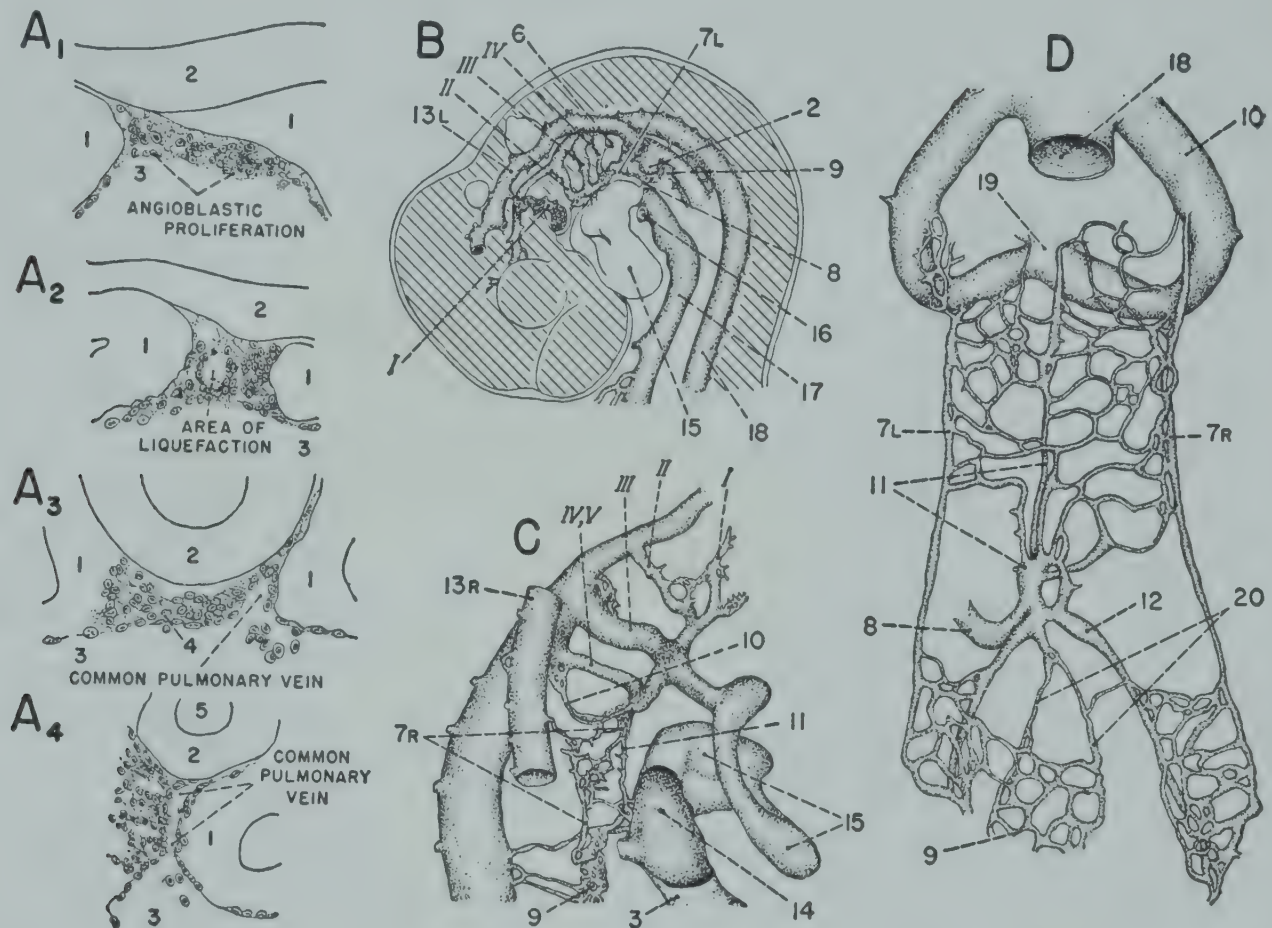
The pulmonary veins condense from an extensive capillary plexus (the so-called splanchnic plexus) that lies caudal to the fourth aortic arch and covers the ventral surface of the gut. This plexus is nonspecific, for its caudal or postcaval portion includes the anlagen of the hepatic system (*Buell*, 1922). According to *Chang* (1931a), the origin of the splanchnic plexus in the chick can be traced back to the 5- to 7-somite stage, when angioblasts can be seen sprouting from the endothelium of the heart tube and spreading over the ventral side of the fore-gut. Subsequently, the endothelium of the heart tube is prolonged dorsally into the dorsal mesocardium, which disappears by the 18- to 20-somite stage in all but its caudalmost portion (see Chapter 4). *Buell* (1922) stated that it is at this time and in this location (the dorsal wall of the sinus venosus extending dorsally toward the lung bud) that angioblasts first proliferate to form the common pulmonary vein (cf. Fig. 255-A<sub>1</sub>); but *Chang* (1931a) regarded the anlage of the pulmonary vein merely as the caudal remnant of the endothelial plate in the dorsal mesocardium. *Federow* (1908) somewhat similarly described the pulmonary vein as arising in the dorsal mesocardium as a longitudinal crestlike endothelial outgrowth of the dorsal wall of the heart at the junction between the sinus venosus and the atrium.

It is possible that the right two thirds of the angioblastic mass gives rise to the endothelium of the lip of the left valve of the sinus venosus (cf. Fig. 255-A<sub>3</sub>) and that the common pulmonary vein is derived from the larger angioblasts of the left one third (*Buell*, 1922). *Chang* (1931a), however, found the lip of the left sinus valve to be of mesothelial and mesenchymal origin.

The lumen of the common pulmonary vein is produced either by central liquefaction in the angioblastic mass (*Buell*, 1922) or by extension of the lumen of the sinus venosus (*Fedorow*, 1908, 1910) through separation of the double layer of endothelium (*Chang*, 1931a). Liquefaction occurs before the vein opens into the sinus venosus (cf. Fig. 255-A<sub>2</sub> and A<sub>3</sub>), but



it also occurs afterward, in regions where no definite blood vessels form (*Chang, 1931a*). The opening of the common pulmonary vein into the sinus venosus is originally left of the opening of the sinus venosus into the primitive atrium of the heart. The interatrial septum is formed between the sinoatrial opening and the mouth of the pulmonary vein, hence the latter comes to empty into the left atrium.



**Fig. 255.** Successive stages in the differentiation of the common pulmonary vein ( $A_1$ ,  $A_2$ ,  $A_3$ ,  $A_4$ ) and other pulmonary vessels ( $B$ ,  $C$ ,  $D$ ) in the chick embryo. (Modified from Buell, 1922.)

$A_1$ , midsagittal section at 20 somites;  $A_2$ , sagittal section at 23 somites. Beginning of differentiation of pulmonary vein indicated by liquefaction of a portion of the proliferation;  $A_3$ , cross section, at 24 somites, earliest stage at which the common pulmonary vein is established. Angioblasts extend from the common pulmonary vein over the ventral surface of the gut;  $A_4$ , sagittal section at 31 somites;  $B$ , pulmonary circulation in the 55-hour chick embryo. The dorsal and ventral primordia of the pulmonary arches are indicated only by blind pouches extending from the angle of the aorta and the combined fourth and fifth aortic arches ( $\times 10$ );  $C$ , right half of the vascular tree in the 60-hour embryo. A portion of the wall of the sinus venosus has been removed to show the opening of the common pulmonary vein into the sinus ( $\times 40$ );  $D$ , dorsal view of the pulmonary system dissected from an injected 85-hour chick embryo ( $\times 40$ ). All definitive vessels are well established; the temporal cranial tributary of the common pulmonary vein is at its greatest and latest stage of development.

1, dorsal mesocardium; 2, lung bud; 3, sinus venosus; 4, lip of left valve of sinus venosus; 5, bronchus; 6, anlage of pulmonary arch; 7L, 7R, left and right pulmonary arteries; 8, pulmonary vein; 9, postcaval portion of the splanchnic plexus; 10, sixth or pulmonic aortic arch; 11, cranial tributary of the common pulmonary vein; 12, right lobar branch of common pulmonary vein; 13L, 13R, left and right anterior cardinal veins; 14, orifice of common pulmonary vein; 15, heart; 16, duct of Cuvier; 17, vitelline vein; 18, dorsal aorta; 19, ventral aorta; 20, caudal tributary of pulmonary vein.



At the stage of 31 somites (cf. Fig. 255-A<sub>4</sub>), the common pulmonary vein of the chick is complete and patent (*Buell, 1922*). It runs from the sinus venosus to the lung bud, and its course, therefore, is directly dorsalward (cf. Fig. 255-B). The entire splanchnic plexus of capillaries drains into the common pulmonary vein, which divides the plexus into cephalic and caudal (postcaval) portions. Before the seventy-second incubation hour (*Squier, 1916*), most of these capillaries condense into larger tributaries; the right and left pulmonary veins, two cranial tributaries anastomosing with the pulmonary arteries (cf. Fig. 255-C), and two caudal tributaries from the more posterior capillaries of the postcaval plexus. The caudal tributaries have disappeared by the 96-hour stage, at which time the cranial tributaries begin to lose their connections with the pulmonary arteries (*Squier, 1916*).

The pulmonary arteries are present before the pulmonary (sixth aortic) arches have formed (*Kastschenko, 1887*). The arteries arise within the portion of the splanchnic capillary plexus that is proliferated from the ventral aorta, and they are carried away from the latter as the ventral primordia of the pulmonary arches grow dorsalward to meet the downgrowing dorsal primordia (*Buell, 1922*). In this way, the pulmonary arteries become branches of the pulmonary arches. Anomalous connections sometimes occur (*Krassnig, 1913a*).

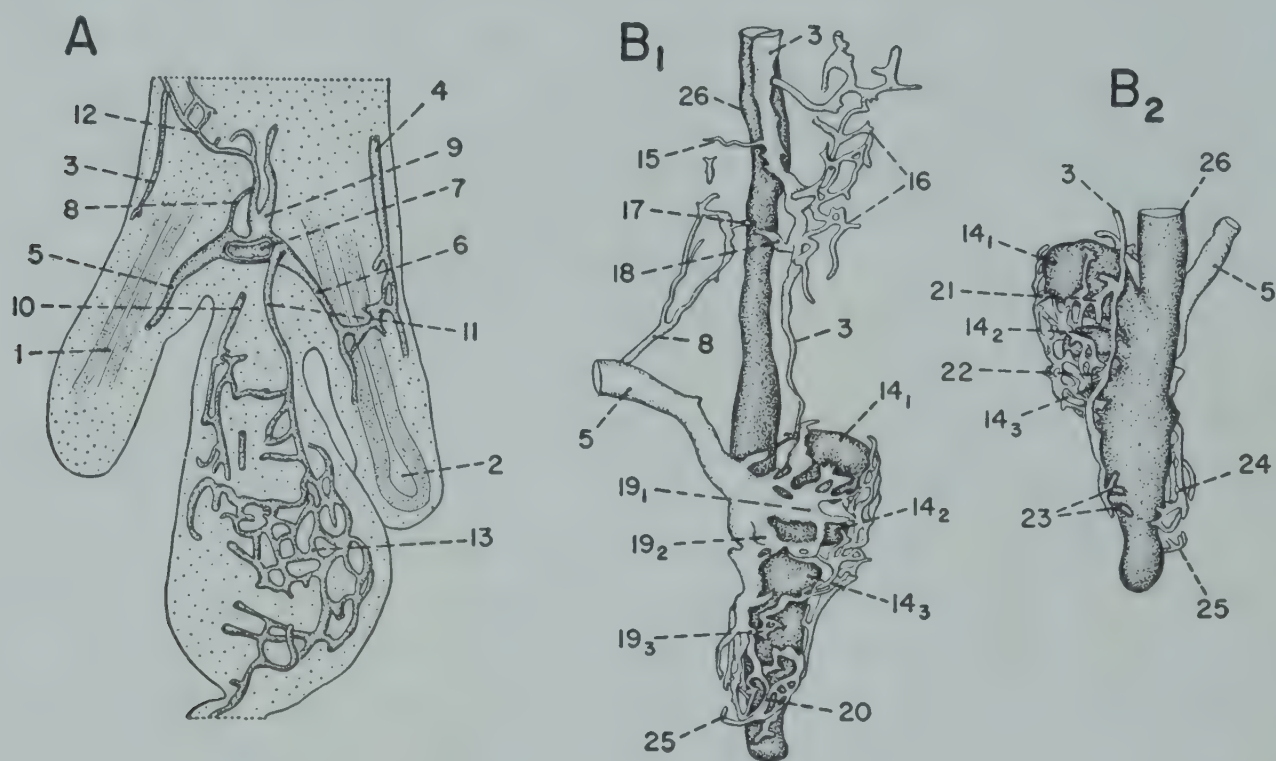
The pulmonary arteries and the pulmonary arches become patent simultaneously at the 35-somite (60-hour) stage (*Buell, 1922*), when the arteries can be traced distally to the lung buds (cf. Fig. 255-B). Here they form anastomoses with the pulmonary veins (Fig. 256-A). Figures 256-B<sub>1</sub> and B<sub>2</sub> indicate the development reached by the chick embryo's pulmonary circulation after 96 hours of incubation, when the lung rudiment consists of three pouches. As the lung becomes increasingly lobular, it is more richly supplied with vessels, and the pulmonary arteries enlarge greatly.

By the end of the seventh day, a septum originating between the fourth and sixth aortic arches has divided the truncus arteriosus (cf. Fig. 240-B) into the aortic and the pulmonic trunk. The latter is the common trunk of the pulmonary arteries. The septum of the truncus arteriosus is continuous with the interventricular septum and develops in such a way that the pulmonic trunk leads off from the right ventricle (see Chapter 9).

The definitive histological structure of the pulmonary artery resembles that of a peripheral muscular artery except for the presence of an outer zone of longitudinal muscle fibers. In the 11-day chick embryo, the wall of the pulmonary artery consists of four layers of cells, outside of which there are five or six widely separated layers of cells destined for the adventitia. Fine elastic fibers are seen in all layers at the 13-day stage. At the 16-day stage, the tunica media is composed of three inner layers of transverse muscle fibers and three outer layers of longitudinal muscle fibers. Collagen fibers



have appeared between the adventitial layers. By the twenty-first day, there are five or six transverse muscle layers, and collagen fibers as well as elastic fibers are present in the longitudinal muscle layers. The adventitia has increased to ten cell layers and is thicker than the entire tunica media (Hughes, 1943).



**Fig. 256.** Two stages in the development of pulmonary circulation in the chick. (Redrawn with modifications after Squier, 1916.)

A, frontal section of the injected 72-hour chick; B<sub>1</sub>, medial and B<sub>2</sub> lateral views of the left lung rudiment and accompanying blood vessels in the reconstructed 96-hour chick. All  $\times 35$ .

1, 2, left and right lung rudiments; 3, 4, left and right pulmonary arteries; 5, 6, left and right pulmonary veins; 7, mouth of common pulmonary vein; 8, 9, left and right median cranial tributaries to common pulmonary vein; 10, 11, left and right caudal tributaries to common pulmonary vein; 12, lateral branch of artery anastomosing with left median cranial vein; 13, plexus surrounding gut; 14<sub>1</sub>, 14<sub>2</sub>, 14<sub>3</sub>, first, second and third lung pouches; 15, blind ventrally directed arteriole; 16, dorsal plexus of artery; 17, branch from the median cranial tributary of the pulmonary vein; 18, ventrally directed arterial branch which probably anastomosed previously with the dorsally directed vessel (17); 19<sub>1</sub>, 19<sub>2</sub>, 19<sub>3</sub>, veins draining first, second, and third lung pouches; 20, distal anastomosis between artery and vein; 21, arterial branch supplying first lung pouch; 22, arterial branch supplying second and third lung pouches; 23, lateral blind arterial pouch; 24, blind distal termination of vein; 25, blind distal arterial termination; 26, left bronchus.

### The Coronary Vessels

It has been observed (Spalteholz, 1923) that coronary arteries first become visible in the 9.0-mm. (approximately 4- to 5-day) chick embryo. They are found in the coronary sulcus and in the apical portion of the anterior longitudinal sulcus. In the 10.6-mm. embryo, the entire epicardium is richly invested with blood vessels of various sizes which can be seen clearly in serial mounts of the sectioned heart.



In histological structure, the coronary arteries are of the peripheral muscular type. At 13 days, their walls consist of only four layers of undifferentiated cells without definite orientation. The definitive structure is emergent at 16 days (*Hughes, 1943*).

### The Vessels of the Abdominal Region

The vascularization of the abdominal region, like that of other areas, involves the successive establishment and obliteration of primitive vessels. Particularly far-reaching changes occur in the venous system, since veins predominate over arteries during the early stages of development. As the definitive vessels develop, many primordial channels disappear completely or in part. Among such transitory vessels are the umbilical, subcardinal, and omphalomesenteric veins, as well as the posterior cardinal veins, whose origin has already been discussed. These vessels participate in the establishment of the vascular system of the liver, kidneys, and pelvic region, a system which is ultimately drained through the posterior vena cava (postcaval vein).

#### *The Subintestinal Veins*

The subintestinal veins are temporary vessels which appear in connection with the drainage of the tail, the leg buds, and the allantois and disappear when their function is taken over by other vessels. In the chick embryo, they are not clearly recognizable before the sixtieth hour of incubation. At the 72-hour stage, it can be seen that a short vessel lying in the midline of the tail divides into two branches. These proceed craniad on either side of the gut, collecting venous blood from the posterior part of the body, including the leg buds. After a short course, the bilateral vessels fuse into a single stem, the main trunk of the subintestinal vein, which continues forward ventral to the closed hind-gut before dividing again into a right and left branch, of which the left is the larger. These branches, which have connections with the vessels of the allantois, run craniad on either side of the open gut to the level where the omphalomesenteric veins enter the embryo. Here the right branch empties into the right omphalomesenteric vein and the left branch into the posterior vitelline vein shortly before the latter joins the left omphalomesenteric vein. As the closed portion of the hind-gut lengthens from posterior to anterior, the subintestinal veins elongate and become continually narrower. New and easier drainage channels are established, with the result that the posterior cardinal vein receives the blood from the caudal region and the leg buds and the umbilical veins drain the allantois. The subintestinal veins atrophy and disappear in the course of the fifth day of incubation (*Hochstetter, 1888; Grafe, 1906*). Probably the smaller right vein degenerates before the left, for a solitary left vein, still connected with the allantoic circulation, has been seen linking the



anastomosed umbilical veins and the left omphalomesenteric vein in a 104-hour duck (*Anas platyrhynchos*) embryo, a 97-hour grouse (*Bonasa umbellus*) embryo, a 5.0-mm. tern (*Sterna hirundo*) embryo, and a 7.2-mm. ostrich (*Struthio camelus*) embryo (Kimball, 1928).

### *The Umbilical Arteries and Veins*

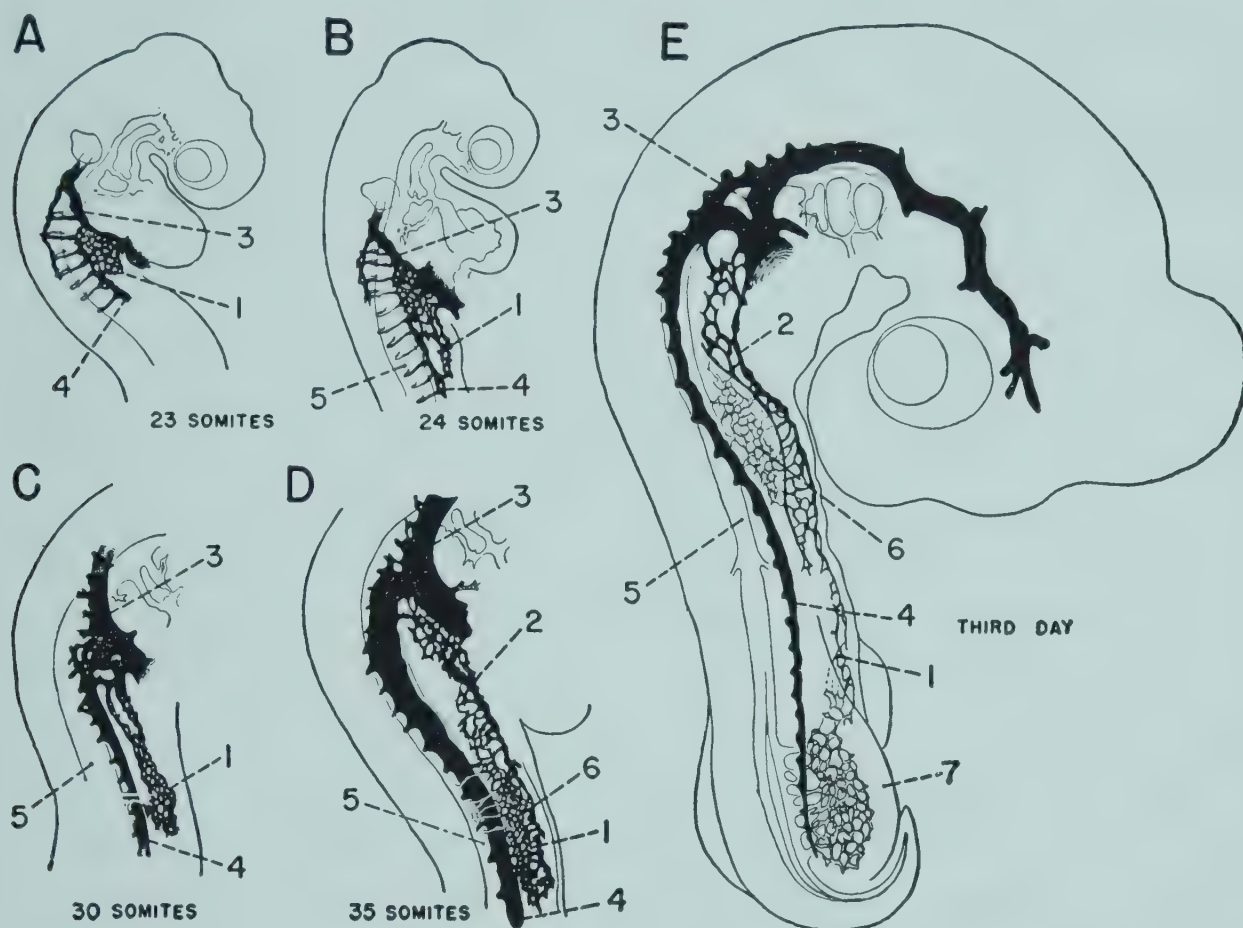
The primitive umbilical arteries of the chick embryo appear at the end of the second incubation day as ventral branches of the still paired caudal aortae. They proceed on either side of the hind-gut to the primordium of the allantois. Somewhat later, when the gut is suspended by the dorsal mesentery, the umbilical arteries pass into the mesentery and, as before, course close to the gut into the ventral body wall and thence to the allantois. During the latter part of the third day, a second pair of large ventrally directed vessels can be seen at approximately the same level as the umbilical arteries; but these vessels are located in the body wall, one on each side, and are close to the lateral borders of the body cavity. They soon anastomose with the umbilical arteries in the ventral body wall where the latter becomes continuous with the dorsal wall of the allantoic stalk. In sections, the body cavities, which are closed ventrally at this level, now appear to be encircled by arterial rings. The medial portions of the arterial rings (that is, the primitive umbilical arteries) disappear, and the lateral portions persist as the definitive umbilical arteries (Duval, 1889; Hochstetter, 1890a). It is apparent at the 90-hour stage that the latter are branches of the sciatic arteries (Miller, 1903), which supply the leg buds.

The anterior portion of each umbilical vein arises from the mesh of capillaries which, bordering the duct of Cuvier, participates in the formation of the posterior cardinal vein (Evans, 1909b). In the 23-somite chick embryo (Fig. 257-A), the capillaries lying ventral to the posterior cardinal vein on each side start to grow caudad in the somatopleure and, by the 35-somite stage, have reached a level posterior to the wing bud (cf. Fig. 257-B, C, and D). A similar outgrowth of capillaries is now directed cephalad from each posterior limb bud, and the anterior and posterior capillaries of either side meet at the end of the third day (cf. Fig. 257-E). At this time, the upper portions of the umbilical veins are clearly resolving from the capillary net as branches of the ducts of Cuvier. As the lower portions form, they anastomose with the vessels of the allantois. Thus the umbilical veins drain the body wall (Hochstetter, 1888) and the limb buds (Evans, 1909b) before they drain the allantois. Subsequently, the connections with the wing and leg veins are lost, in the manner already described.

At first the right umbilical vein is somewhat larger than the left, but only for a brief period of time. During the chick's fourth incubation day, the anterior portion of the left umbilical vein branches into the left liver



lobe and forms anastomoses with the liver circulation (cf. Fig. 258-D). The left umbilical vein now enlarges greatly, surpassing its counterpart on the right, and loses its connection with the duct of Cuvier (cf. Fig. 258-E) late in the fourth day (*Brouha, 1898b*) or early in the fifth day (*Hochstetter, 1888*). It now empties into the ductus venosus (the common trunk of the omphalomesenteric veins) through a series of small canals; soon, however, it joins with the left hepatic vein to form a common stem. After



**Fig. 257.** Successive stages in the development of the umbilical vein in the chick embryo. (Redrawn with modifications after *Evans, 1909b*.)

A, umbilical vein originating from a capillary plexus located in the angle between the posterior cardinal veins and the duct of Cuvier; B, capillary plexus present in A, extended both longitudinally and laterally; C, capillaries contributing to the formation of the upper portion of the umbilical vein, joined with the differentiating subclavian artery in the region of the future wing bud; D, the well-established umbilical vein which drains the wing bud vessels; E, capillary extensions of the umbilical vein reach as far caudad as the posterior limb bud, designating the future course of the posterior portion of the umbilical vein. All  $\times 15$ .

1, capillaries destined to form the umbilical vein; 2, umbilical vein; 3, anterior cardinal vein; 4, posterior cardinal vein; 5, aorta; 6, wing bud vessels; 7, hindlimb bud.

air breathing begins, the left umbilical vein persists as a median vein lying between the lobes of the liver and draining the subserous tissues of the ventral body wall (*Hochstetter, 1888*).

According to *Brouha (1898b)*, the right umbilical vein shifts its mouth from the duct of Cuvier to the ductus venosus at the same time as the left vein, but at a level anterior to the right lobe of the liver, which has not yet grown as far forward as the left lobe. By the middle of the fifth day,



however, the terminal portion of the right umbilical vein passes through the liver (*Brouha, 1898b*). Later, the anterior part of this vein undergoes atrophy. The direction of blood flow through its posterior portion is then reversed; blood is carried to the allantois from the body wall, rather than away from the allantois. Eventually, the posterior portion also disappears (*Hochstetter, 1888*).

*The Omphalomesenteric Veins, the Ductus Venosus,  
and the Hepatic Veins*

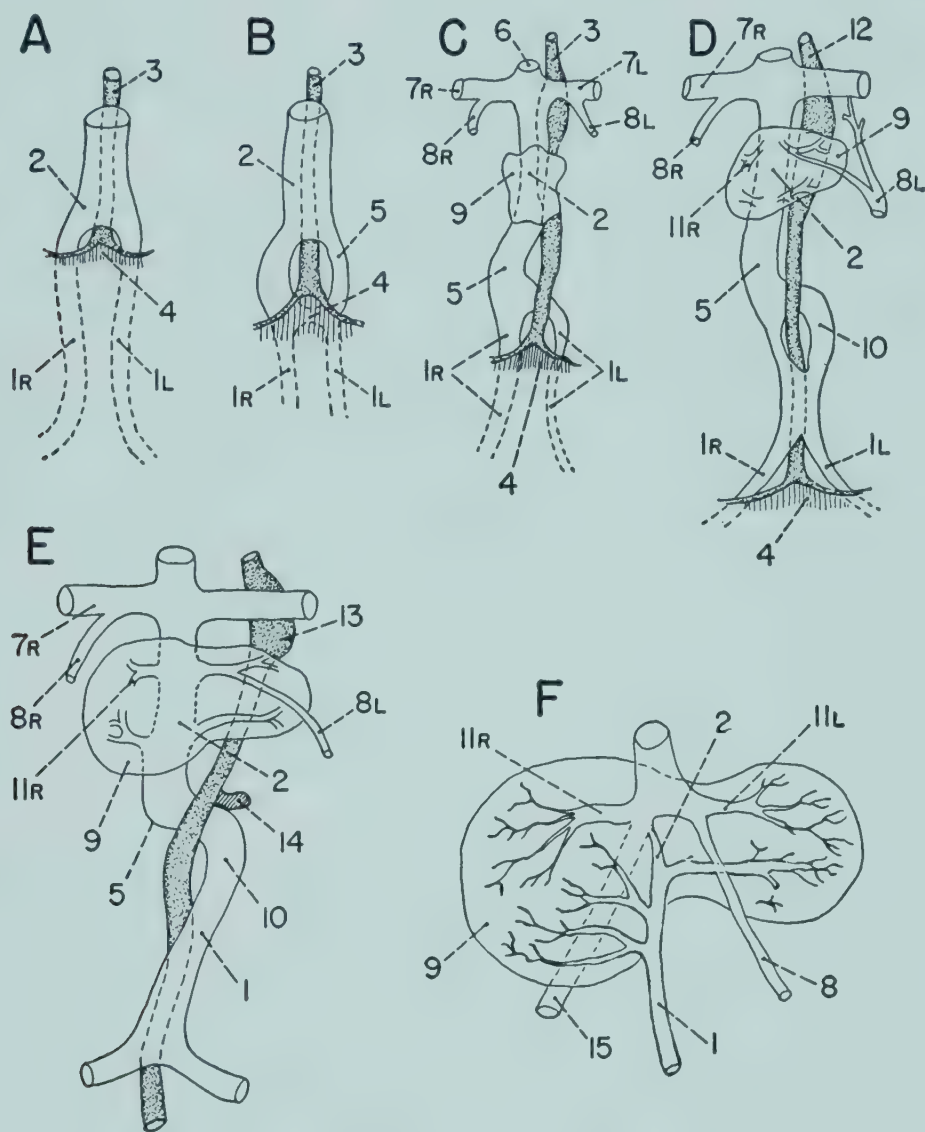
The bilateral vitelline or omphalomesenteric veins are established as extraembryonic vessels oriented perpendicular to the long axis of the embryo. They enter the embryonic area at the level of the anterior intestinal portal, where they join ventral to the gut (Fig. 258-A). Their common stem parallels the long axis of the embryo and empties into the sinus venosus of the heart close to the mouths of the ducts of Cuvier. As the fore-gut closes, the most proximal portions of the omphalomesenteric veins continue to come into apposition and to fuse together, thus elongating their common trunk caudad. At and above the level of the liver anlagen the common omphalomesenteric trunk is termed the ductus venosus (or meatus venosus), which continues into the sinus venosus without visible transition.

After approximately 60 hours' incubation, tissue derived from the chick's liver diverticula becomes inextricably associated with endothelial sprouts given off by the ductus venosus. The fore-gut posterior to the hepatic region arches ventrally between the omphalomesenteric veins. These veins now fuse with each other at a single point dorsal to the fore-gut, thus forming a ring around the gut caudal to the liver diverticula (Fig. 258-B). Between the sixty-fourth and sixty-eighth hours of incubation (*Brouha, 1898b*), the portion of the ring composed of the left omphalomesenteric vein disappears (Fig. 258-C). By the eightieth hour a second and more posterior ring develops around the intestine (Fig. 258-D), which, of course, is now longer. The site of the second anastomosis is ventral to the fore-gut and posterior to the primordium of the pancreas. Formed by the eightieth hour, this second ring also quickly disappears through the atrophy of its right half (*Hochstetter, 1888*). Because of the formation and disappearance of these rings, the omphalomesenteric trunk swerves dorsally around the gut from right to left (Fig. 258-E).

Before the eightieth hour of incubation, most of the blood entering the ductus venosus from the omphalomesenteric veins and their common trunk flows directly to the heart; only a small part is diverted through the liver anlagen. After this time, however, liver development proceeds rapidly, and the ductus venosus sends continually larger branches into the liver. Large afferent and efferent veins appear in both the right and the left lobes of



the liver. During the fifth and sixth days, the ductus venosus gives off increasingly numerous hepatic branches, while simultaneously growing narrower (Fig. 258-F). It finally breaks down completely into the network of the hepatic circulation and disappears by the end of the seventh day (*Hochstetter, 1888*). Its extreme anterior end persists, however, as the root of the posterior vena cava.



**Fig. 258.** Diagrams illustrating the developmental history of the omphalomesenteric, umbilical, and hepatic veins, and the ductus venosus of the chick embryo. (Redrawn with modifications after *Hochstetter, 1888*.)

A, the 58-hour stage; B, the 65-hour stage; C, the 75-hour stage; D, the 80-hour stage; E, the 100-hour stage; F, the 140-hour stage.

1R, 1L, right and left omphalomesenteric veins; 2, ductus venosus; 3, fore-gut; 4, anterior intestinal portal; 5, first venous ring; 6, sinus venosus; 7R, 7L, right and left ducts of Cuvier; 8R, 8L, right and left umbilical veins; 9, liver; 10, second venous ring; 11R, 11L, right and left sides; 12, esophagus; 13, stomach; 14, pancreas; 15, inferior vena cava.

Posterior to the liver, the common omphalomesenteric vein has meantime (during the fourth and fifth days) developed a branch, the mesenteric vein, which lies dorsal to the gut and posterior to the pancreas. This vein increases in size and importance and, with the omphalomesenteric trunk, gives rise to the stem of the hepatic portal vein (*Hochstetter, 1888*). In the adult, the hepatic portal vein is a branch of the common mesenteric vein



(*Hochstetter*, 1894) or is anastomosed with it (*Spanner*, 1925). On the eighth day, the coccygeo-mesenteric vein (a branch of the common mesenteric vein) may be seen as a small vessel along the dorsal surface of the hind-gut (*Miller*, 1903). At some later time, the coccygeo-mesenteric vein joins the anastomosed posterior cardinal veins caudal to the kidney. In this way, the renal and the hepatic portal systems are connected (*Hochstetter*, 1894).

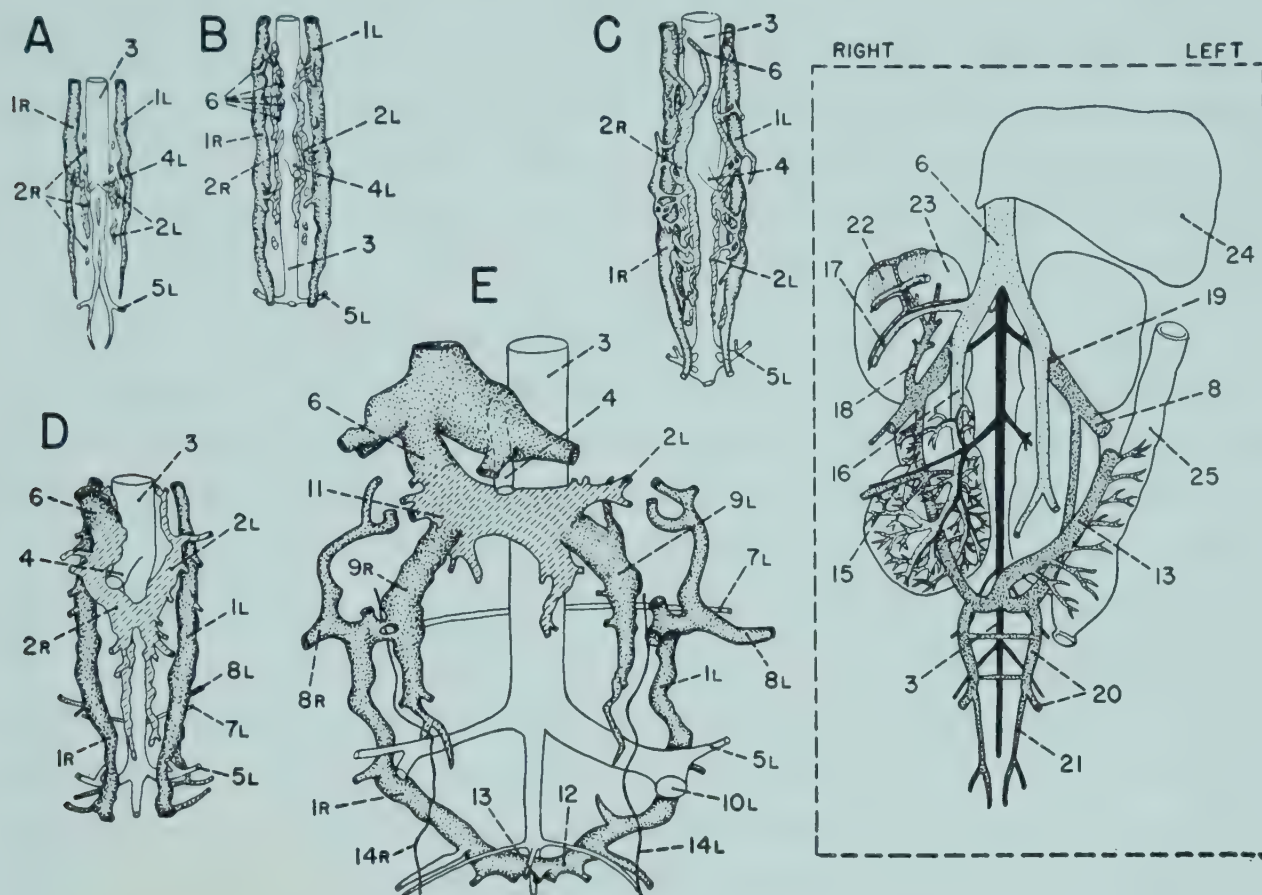
*The Subcardinal Veins, the Posterior Vena Cava,  
and the Renal Vessels*

The subcardinal veins begin to form ventromedial to the posterior cardinal veins before the latter are complete in their caudal portion. Anlagen of the subcardinal veins become visible in the chick embryo during the last hours of the second day of incubation; by the forty-eighth hour, they are present from the anterior end of the Wolffian duct almost to the level of the omphalomesenteric arteries (*Grafe*, 1906). These anlagen consist of sprouts, at first solid and then hollow, which are given off from the posterior cardinal veins (Fig. 259-A) and which form longitudinal anastomoses with one another (*Miller*, 1903; *Grafe*, 1906). The sprouts grow dorsally and laterally around the Wolffian duct, and the longitudinal anastomoses form ventral to it. In the 2.5-day embryo, small ventrolateral branches of the aorta anastomose with the subcardinal veins. Blood can now pass from the aorta to the subcardinal veins and thence, through additional anastomoses, to the posterior cardinal veins. At the end of the third day, the subcardinal vein forms anastomoses with the subintestinal vein, and blood from the legs, the tail, and (via the caudal or posterior vitelline vein) the yolk sac is thus led into the subcardinal veins and from these veins to the posterior cardinal veins. The subcardinal veins also collect blood from the mesentery and the liver region. Since the connections between the subcardinal and the posterior cardinal veins (except anteriorly) lead into and through the circulation of the mesonephros, the subcardinal veins are the earliest renal portal veins. With the atrophy of the subintestinal veins at the beginning of the fifth day, the blood formerly carried by them flows directly into the posterior cardinal veins, which have meantime grown into the lower portion of the body (*Grafe*, 1906).

By this time, the inferior vena cava has begun to form. This vessel, taking origin from the postcaval plexus, can first be seen at about the ninetieth incubation hour extending discontinuously in the caval mesentery from the ductus venosus, past the liver primordia, to the mesonephros; it ends just anterior to the level where the omphalomesenteric arteries arise from the aorta (cf. Fig. 259-B). In the hepatic region, it forms partially through the confluence of the sinusoids or venous spaces of the liver. In the 4-day embryo, the postcaval vein is seen as a continuous, luminate



vessel which originates from the ductus venosus below the right hepatic veins and passes through the right lobe of the liver to join the right subcardinal vein at a point not far below the anterior junction of the latter with the right posterior cardinal vein. The right subcardinal vein thus be-



**Fig. 259.** The development of the renal portal system in the avian embryo. (Redrawn with modifications A to D, after Miller, 1903; E and Insert, after Spanner, 1925.)

A, the initial development of the subcardinal veins in the sparrow (*Passer domesticus*) embryo, corresponding to a chick incubated 60 to 70 hours; B, initial development of the inferior vena cava in the sparrow embryo, corresponding to a chick incubated 90 hours or less; C, an early stage in the development of the inferior vena cava in the chick embryo, incubated 90 hours; D, the inferior vena cava and the posterior cardinal and subcardinal veins in a 5-day chick embryo; E, the same veins as in D, together with the newly formed kidney veins, in the sparrow embryo, corresponding to a chick incubated 14 days. All  $\times 10$ .

*Insert:* schematic representation of the vascular system in the adult avian kidney.

1L, 1R, left and right posterior cardinal veins; 2L, 2R, left and right subcardinal veins; 3, aorta; 4L, left omphalomesenteric artery; 5L, left sciatic artery; 6, inferior vena cava; 7L, left iliac artery; 8L, 8R, left and right external iliac veins; 9L, 9R, left and right great renal veins (vena renalis magna); 10L, left umbilical artery; 11, subcardinal portion of inferior vena cava; 12, anastomosis between posterior cardinal veins; 13, coccygeo-mesenteric vein; 14L, 14R, left and right ureter; 15, renal portal vein; 16, efferent renal vein of lower kidney lobe; 17, efferent renal vein of upper kidney lobe; 18, afferent vein of upper lobe; 19, valve; 20, hypogastric vein; 21, common coccygeal vein; 22, capillaries; 23, kidney; 24, liver; 25, intestine.

comes a continuation of the postcaval vein. Soon afterward (during the fifth incubation day), the right and left subcardinal veins anastomose at a level posterior to the omphalomesenteric arteries (cf. Fig. 259-C), and thus the posterior portion of the left subcardinal vein is added as a further



continuation of the postcaval vein (*Hochstetter*, 1888, 1894; *Miller*, 1903).

The direction of blood flow is now reversed; instead of passing from the subcardinal veins to the posterior cardinal veins, the blood proceeds from the latter through the mesonephroi to the subcardinal veins and thence to the inferior vena cava (*Grafe*, 1906; *Waldeyer*, 1931). The vena cava becomes greatly enlarged (*Hochstetter*, 1888, 1894; *Miller*, 1903) and soon appears as a direct continuation of the ductus venosus.

During the fifth incubation day, the metanephroi begin to form. The entire venous circulation of the renal region now passes through both embryonic kidneys. On the seventh day, lateral branches of the posterior cardinal vein can be seen to communicate with the sinusoids of the mesonephros, which in turn are connected, by means of venous loops, with the subcardinal veins (*Waldeyer*, 1931). The subcardinal veins thus drain both the mesonephroi and the metanephroi.

As the mesonephroi begin to atrophy, so also do the cephalic portions of the subcardinal veins—that is, the left subcardinal anterior to the anastomosis, and the right subcardinal anterior to its junction with the vena cava. According to *Miller* (1903), both veins posterior to the anastomosis also disappear, so that the subcardinal veins are perhaps represented in the adult merely by the bifurcation of the inferior vena cava into right and left common iliac veins. *Spanner* (1925), however, expressed doubt that so large a part of the subcardinal veins retrogresses. The genital and suprarenal veins, emptying into the postcaval vein, originate as branches of the subcardinal veins (*Miller*, 1903).

The posterior portions of the subcardinal veins form branches which will remain as the permanent efferent veins of the metanephros (*Miller*, 1903). Small branches of the efferent veins may be seen in the forming kidney lobules on the eighth incubation day; by the tenth day, two large branches of the subcardinal vein, one ventrolateral and the other dorsolateral, pass to each metanephros (*Waldeyer*, 1931). *Spanner* (1925) was inclined to regard the efferent veins of the kidney as the persisting subcardinal veins.

Meantime, the anterior portions of the posterior cardinal veins disappear. Their retrogression begins soon after the time when the subcardinal veins anastomose and the direction of blood flow is reversed. According to *Spanner* (1925), part of the posterior cardinal vein cephalic to the junction of the external iliac vein persists as the afferent vein of the upper lobe of the kidney, although *Miller* (1903) believed a vein in a corresponding location to be a new formation and ascribed to it an efferent function. *Spanner* (1925) suspected that the efferent vein of the upper lobe is a persisting portion of the subcardinal vein. It lies ventral and medial to the afferent vein of the upper lobe (cf. Fig. 259—Insert).

The caudal portions of the posterior cardinal veins anastomose with each



other in the tail region on the sixth day (Miller, 1903) and, somewhat later, with the coccygeo-mesenteric vein. The anastomosis gradually occupies a more anterior position, until, on the eleventh day, it lies immediately caudal to the posterior kidney lobe (cf. Fig. 259-E). At some time between the 9.5-day stage (Waldeyer, 1931) and the 14-day stage (Miller, 1903), the efferent veins of the posterior kidney lobe form an anastomosis with the posterior cardinal veins at the level of the external iliac veins (cf. Fig. 259-E). Hochstetter (1888, 1894), Miller (1903), and others presumed that the formation of this anastomosis destroyed the renal portal system. Spanner (1925), however, showed that the site of the anastomosis is marked in the adult bird by a valve, which prevents direct flow of blood from the external iliac and afferent renal veins into the common iliac and postcaval veins. Thus a renal portal system is maintained permanently. Spanner (1925) also observed that the coccygeo-mesenteric vein brings blood toward the kidney instead of carrying it from the kidney to the liver, as was believed by many of his predecessors. In the adult, the blood from the posterior intestine (via the coccygeo-mesenteric veins), the pelvis (via the common coccygeal and hypogastric veins), and the legs (via the internal and external iliac veins) is brought to the kidneys by the afferent renal veins. After passing through the capillary system of the kidneys, it is carried by the efferent renal veins into the inferior vena cava and thence to the heart (cf. Fig. 259—Insert).

The definitive arteries of the kidney start to develop later than the veins. At the end of the third day, two or three small arteries per segment branch from the aorta to the mesonephros. These arteries and their branches are the vasa afferentia of the mesonephric glomeruli. They empty into the vasa efferentia, which are the anastomosing vessels between the cardinal and subcardinal veins (Grafe, 1906). In the 6.5-day chick embryo, only five mesonephric arteries are seen. Sprouts from the two lowest of these enter the cranial portion of the metanephros; this portion will become the upper lobe of the kidney. At the 7-day stage, the caudal part of the metanephros receives a partially solid arterial sprout given off ventrally from the sciatic artery. The two caudalmost mesonephric arteries and the cranial metanephric artery have become larger, whereas the three most cranial mesonephric arteries show the first signs of atrophy. A day later, the artery of the upper metanephros branches from the lowest mesonephric artery. The posterior metanephric artery has grown dorsally and has divided into a dorsal and ventral branch, and the dorsal branch has divided further into caudal and cranial branches. The metanephric arteries and arterioles accompany branches of the subcardinal vein. At this time (the 8-day stage), the lobules of the kidney are beginning to differentiate, and the efferent lobular veins and the central lobular arteries are seen centrally in the lobules, the tubules and afferent veins peripherally. Shortly after the completion of the



tenth incubation day, the venous circulation of the metanephros is superseded by arterial circulation (*Waldeyer, 1931*).

Kum  (1952), investigating self-differentiation of the venous system of renal circulation of 6- and 8-day chick embryos in which the development of the mesonephros had been experimentally arrested, found renal venous circulation can develop almost normally despite absence of the mesonephros until the sixth incubation day. Accelerated development of the venous system, exceeding by far the normal, follows up to the stage of the start of degeneration of the mesonephros and the almost complete differentiation of the metanephros.

### *The Coeliac Artery*

The coeliac artery appears in the chick embryo at some time between the fiftieth and sixtieth hours of incubation. Initially, it is a midventral branch of the aorta arising in the vicinity of the omphalomesenteric artery, but its point of origin is shifted during the second half of the third day to the right lateral wall of the aorta (*Bremer, 1924a*).

This shifting of the proximal end of the coeliac artery is not an autonomous migration; the vessel is literally pulled to its definitive position by sympathetic nerve fibers originating from the primary ganglionic chain of the right side. These fibers reach and enmesh the root of the coeliac artery, whereas those of the left side fail to contact it. This circumstance is due to the fact that the aorta, at the level of the coeliac root, is slightly to the right of the midline when the outgrowth of sympathetic fibers occurs. The asymmetrical position of the aorta can in turn be traced to the sinistral rotation of the head which takes place in the chick between the thirty-sixth and ninety-sixth hours of incubation, involving progressively more caudal portions of the trunk (*Bremer, 1924a*).

The sympathetic fibers of the right side reach and surround the root of the coeliac artery after 3.5 days' incubation. There rapidly ensues an apparent dorsal withdrawal of the primary sympathetic chains, a process which is actually due to the growth of mesodermal structures between the aorta and the sympathetic trunks (*Bremer, 1926*). In the 4-day embryo, the coeliac artery has been drawn dorsalward and to the right at its proximal end, where unilateral traction is exerted on it by the fibers of the right side. Farther caudally, where it is reached by fibers of both sides, the artery returns to the midline, as can be seen in Fig. 260-A at the 5-day stage. The omphalomesenteric artery is similarly affected (cf. Fig. 260-B). By the use of operational procedures, *Bremer (1926)* was able either to prevent the normal body asymmetry or to retard the growth of the right sympathetic fibers in several embryos; in these specimens, the root of the coeliac artery retained its position in the midline ventral to the aorta.

After returning to the midline, both the omphalomesenteric and the



coeliac arteries run caudally in the mesentery before branching to the yolk sac and gut, respectively. The two main branches of the coeliac artery are the gastric and hepatic arteries. The gastric branch passes in the mesentery across the embryo to the stomach, far to the left.

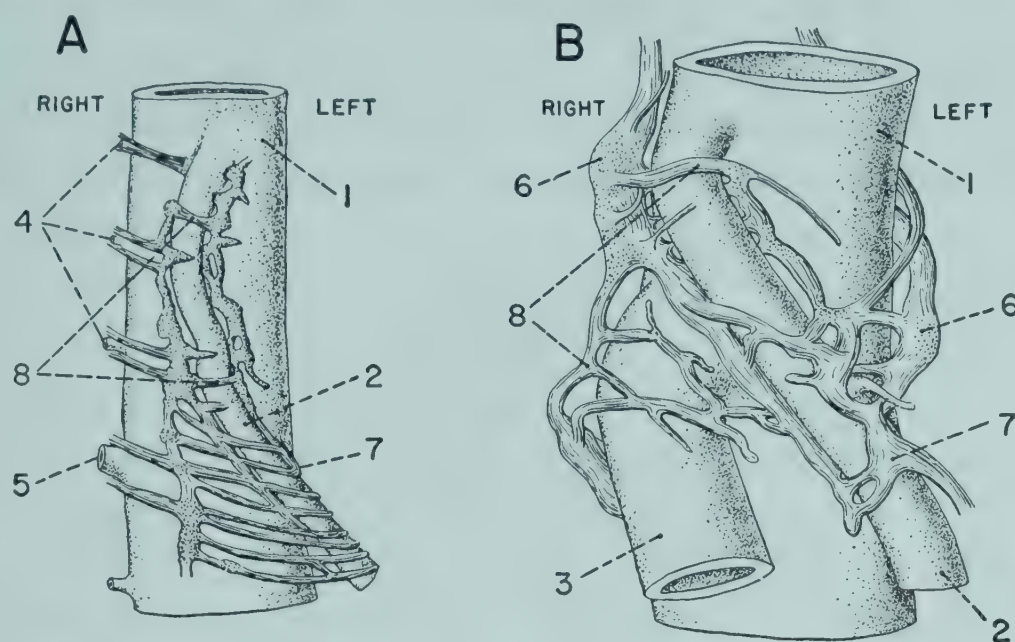


Fig. 260. Interrelationship between nerve fibers and the coeliac artery in the developing chick. (Redrawn with modifications after Bremer, 1924b.)

A, at 5 days 1 hour, dextral view of the portion of the aorta giving rise to the coeliac artery, showing the artery arising from the right wall and pulled dorsally by the traction of the nerve fibers until, posterior to the level of the subclavian artery, it is held in the dorsomedian line by nerve fibers from both sides; B, at 11 days, ventrolateral aspect of portions of the coeliac and vitelline arteries and the aorta, showing extensions of the periaortic nerve fibers from the left and right ganglionic chains encircling the portion of the coeliac artery displaced dorsally. Both  $\times 38$ .

1, aorta; 2, coeliac artery; 3, omphalomesenteric artery; 4, severed stumps of dorsal segmental arteries; 5, subclavian root; 6, sympathetic ganglionic chain; 7, sympathetic nerve fibers of left side; 8, sympathetic nerve fibers of right side.

### The Vessels of the Spinal Cord

The vessels of the spinal cord are derived from segmental branches of the aorta and the posterior cardinal veins. During the chick's third incubation day, the cardinal veins direct sprouts toward the spinal cord in each intersomitic space (cf. Fig. 240-B<sub>3</sub>). These sprouts are the anlagen of the segmental spinal veins. At the same time, the stems of the intersomitic arteries each send a sprout directed dorsally, lateral to the spinal cord (cf. Fig. 240-B<sub>1</sub> and B<sub>3</sub>); thus arise the segmental spinal arteries. These anastomose with the spinal veins (Fig. 261-A; cf. Fig. 240-A<sub>2</sub> and B<sub>1</sub>) on each side of the spinal cord (Grafe, 1906; Sabin, 1917b; Hughes, 1934).

The subsequent development of the chick's spinal cord vessels has been well described by Sterzi (1904). Somewhat earlier than the 72-hour stage, the segmental arteries start to send rami medialward into the mesenchyme between the spinal cord and the chorda dorsalis (Fig. 261-A; cf. Fig. 240-B<sub>1</sub>). During the second half of the fourth day, longitudinal anastomoses



begin to connect the medial ends of these rami on either side (cf. Fig. 261-B). The vessels formed by these anastomoses have been termed the "primitive arterial tracts" (Sterzi, 1904) and represent the paired anlagen of the eventually single anterior spinal artery. At the 84-hour stage, the primitive arterial tracts send dorsal branches into the substance of the spinal cord; these are the first endoneural vessels of the spinal cord and will give rise to the paired longitudinal endoneural sinuses. Each of the arterial tracts also gives off numerous cranial and caudal rami which form

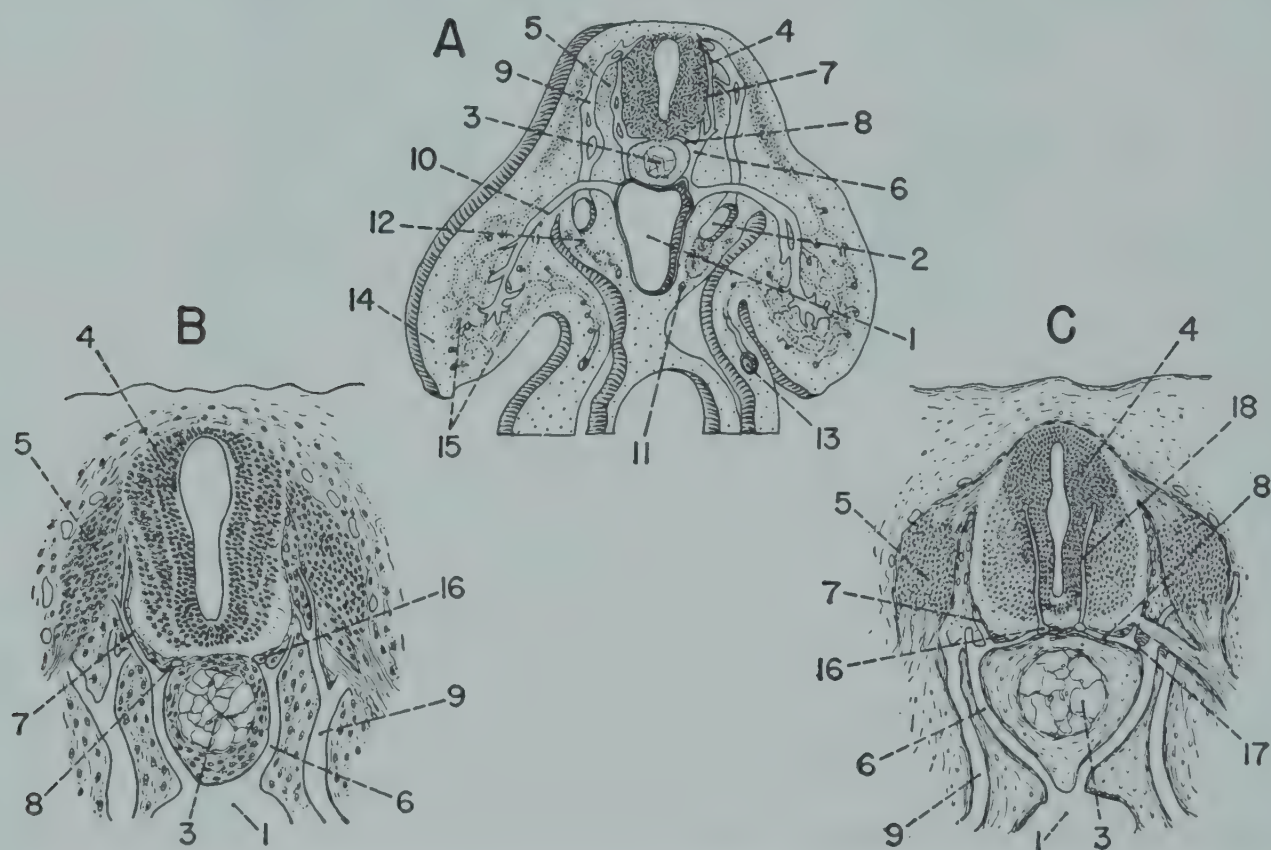


Fig. 261. The development of the vessels of the spinal cord in the chick embryo, as seen in cross sections. (Redrawn with modifications A, from Evans, 1909a; B and C, from Sterzi, 1904.)

A, at the 34-somite (58 hours) stage at the level of the anterior limb buds, that is, through the eighteenth interspace ( $\times 20$ ); B, after 88 hours' incubation ( $\times 65$ ); C, after 5 days' incubation ( $\times 35$ ).

1, aorta; 2, posterior cardinal vein; 3, chorda; 4, spinal cord; 5, spinal ganglion; 6, dorsal segmental artery; 7, dorsal ramus of dorsal segmental artery; 8, medial ramus of dorsal segmental artery; 9, dorsal segmental vein; 10, subclavian artery; 11, subcardinal vein; 12, nephric vein; 13, umbilical vein; 14, limb bud; 15, capillaries of limb bud; 16, primitive arterial tract (anterior spinal artery); 17, vertebromedullary artery; 18, dorsal branch of primitive arterial tract.

a network of anastomosing capillaries over the ventral surface of the spinal cord (Sterzi, 1904; Hughes, 1934). This network drains into the segmental veins through ventral rami of the latter. Meantime, the dorsal rami of the segmental arteries (that is, the more distal portions of the arteries, lateral to the spinal cord, as distinct from the more proximal portions, lateral to the chorda) have divided ventral to the spinal ganglia into ganglionic branches and branches to the spinal cord. These branches have formed capillary networks over the ganglia and the lateral surface of the cord, re-



spectively, which feed blood into each segmental vein through rami lying dorsal and ventral to each spinal ganglion. (The ventral rami are formed by the confluence of smaller rami that lie caudal and cranial to each ganglion.)

In the 5-day embryo, both the spinal cord and the anlagen of the vertebrae have grown wider, and the medial rami of the segmental arteries have lengthened correspondingly (Fig. 261-C). The segmental arteries no longer continue directly into their dorsal rami, for the dorsal rami are now given off from the medial rami a short distance from the origin of the latter. In the terminology of Sterzi (1904), the common stem of each medial and dorsal ramus now becomes the vertebromedullary artery. The medial ramus—the direct continuation of the vertebromedullary artery—is the ventral radicular artery, and the dorsal ramus is the dorsal radicular artery; these vessels receive their names from their proximity to the ventral and dorsal roots of the spinal nerves.

In the embryo incubated 5.5 days, the primitive arterial tracts become interconnected by anastomoses that cross the ventral midline. Collateral branches of the vertebromedullary arteries (which are now longer) have anastomosed with the capillary network arising from the primitive arterial tracts. The entire surface of the spinal cord is covered by a capillary net. In the 6.5-day embryo, the capillary net is much denser on the dorsal surface than elsewhere, and its individual vessels are of smaller caliber in this region. The paired vertebral arteries have now been formed by longitudinal anastomoses between the segmental arteries at the level where the vertebromedullary arteries are given off.

The ventral capillary net is much finer after 8 days' incubation than previously, and the primitive arterial tracts are more regular and of smaller caliber. The dorsal radicular arteries now divide into cranial and caudal rami directly ventral to the dorsal roots of the spinal nerves. The anastomosis of the cranial and caudal rami of successive dorsal radicular arteries has formed a lateral arterial tract, which gives off dorsal and ventral rami to the superficial capillary net of the spinal cord. Two days later, the dorsal rami of the lateral arterial tract have in turn sent out cranial and caudal rami which have anastomosed to form a dorsal arterial tract.

In the 10-day embryo, the primitive arterial tracts show the first indications of their eventual consolidation into a single vessel. In the cervical region, irregularly alternating portions of the right and left tracts are beginning to atrophy. These portions have disappeared at the 12.5-day stage as far caudal as the lumbar region. Some of the medial rami that formerly connected the two arterial tracts have become incorporated into the single persisting artery. Due to the atrophy of one or the other of every pair of dorsal rami, each of these rami is now reduced to a single proximal stem branching distally into the paired central arteries, which lie in the median ventral fissure of the spinal cord.



At the 10-day stage, numerous veins and arteries enter and leave the substance of the spinal cord. The anlagen of the meninges are now present, and the ventral venous capillary net consists of two layers. The veins that leave the cord form a delicate ventral perimeningeal layer, from which branches lead to a ventral endorhachidian layer; this lies in a connective tissue layer and corresponds to the earlier ventral venous capillary net. The perimeningeal net of the dorsal surface is not yet well developed. The dorsal endorhachidian net is condensing into the median dorsal endorhachidian sinus and its paired lateral emissaries. The latter are still plexiform and empty into the paired longitudinal vertebral veins by means of short rami passing cranial and caudal to each dorsal spinal nerve root. According to Hughes (1934), the vertebral veins are quite indefinite at 8.5 days and are still rather irregular at 12 days. At the latter time, the middorsal longitudinal sinus is first seen to be extending from the occipital region down the spinal cord. The vertebral veins give off branches to run outward with each nerve root and are connected with the lateral occipital vein between the first and second cervical ganglia.

In the 12.5-day embryo (Sterzi, 1904), the perimeningeal and endorhachidian networks are separated by the perimeningeal space. The ventral endorhachidian sinus is developing in the same manner as its dorsal counterpart. The ventral and dorsal perimeningeal net are drained by the ventral and dorsal radicular veins. The last veins to appear are seen at the 14-day stage coursing parallel to the lateral arterial tracts.

#### *The Intraneural Vessels of the Spinal Cord*

As previously noted, the paired dorsal rami of the primitive arterial tracts penetrate the substance of the spinal cord from its ventral side at the 84-hour stage. In the 4-day chick embryo (Feeney and Watterson, 1946), these penetrating capillaries have grown dorsally to a point level with the roof of the neural canal and are situated just within the lateral boundaries of the ependymal layer. On either side of the spinal cord, longitudinal anastomoses between the dorsal ends of successive capillaries form the longitudinal endoneural sinus. The paired endoneural sinuses of the spinal cord are continuous with the similar endoneural sinuses of the brain.

On the chick's fourth and fifth incubation days, the second group of intraneural vessels appears in the spinal cord. This group consists of four dorsolateral vessels, two on each side, which traverse the white matter and connect the endoneural sinuses with the superficial capillary plexus of the cord. Most of these dorsolateral sprouts seem to originate from the endoneural sinuses, but some possibly grow in from the surface. On the sixth day, the first interconnections between the intraneural vessels are established, and during the next 3 days an intricate plexus of vessels develops entirely within the gray matter of the cord, progressing from ventral to



dorsal. Additional capillaries extending to the superficial plexus arise during this time; in the order of their appearance, these are ventrolateral, lateral, dorsal, and ventral in position. Also, the endoneural sinuses become interconnected dorsal to the neural canal. On the ninth day, a conspicuous capillary penetrates through the dorsal gray column. After the tenth day, the plexus in the gray matter begins to extend into the dorsal gray columns. In the 12-day embryo, plexus formation is spreading into the white matter, and the penetrating vessels are much more numerous. The 16-day intraneural plexus of the cord is exceedingly complex and approaches the adult condition (*Sterzi, 1904; Feeney and Watterson, 1946*).

The intraneural vessels arise in practically the same sequence throughout the major portion of the spinal cord. The vascular pattern formed by them develops at the same rate from the cervical swelling to the posterior boundary of the lumbosacral swelling, but evolves more slowly anterior to the cervical swelling and in the caudal region. Some of the vessels appear in different order at these levels. The most posterior part of the cord contains no vessels within its walls during the first 16 days of incubation (*Feeney and Watterson, 1946*).

## LYMPHATIC VESSELS AND LYMPH GLANDS

The vascular system is supplemented by another system of vessels, the lymphatics. The function of the lymphatic vessels is to return to the blood stream part of the plasma which has passed out through the walls of the blood vessels to bathe the tissues as tissue fluid. Once it has entered the lymphatic vessels, this fluid is known as lymph. Lymph from the entire body is emptied into the superior venae cavae, most of it passing through the paired thoracic ducts, which are the largest lymphatic vessels. Lymph thus flows in one direction—from the tissues to the blood stream—and does not circulate as does blood.

Like the blood vessels, the lymphatics are lined with endothelium, but their walls are more irregular than those of arteries and veins. The smallest lymphatic vessels are of approximately the same size as the blood capillaries, but the largest do not approach the caliber of the major blood vessels. Where lymphatic vessels join veins, valves prevent the backflow of blood.

Unlike mammals, birds possess either very few lymph glands or none at all, depending upon the species. When present, lymph glands are situated along the course of lymphatic vessels and are derived from enlargements of the latter. Lymph glands produce lymphocytes and consist of aggregates of lymphatic (lymphoid) tissue. Nodules of lymphatic tissue are also abundantly scattered throughout various organs of birds, as previously indicated in the discussion of white blood cell formation.

In addition to lymphatic vessels and lymph glands, a few species of adult



birds possess pulsating "lymph hearts." These are small, oval, hollow organs, whose walls contain muscle and whose contractions assist the flow of lymph. In many birds, lymph hearts are present only during embryonic life.

In the past, there have been various misconceptions concerning the nature of the lymphatic vessels. Before it was known that lymphatics were lined with a definite layer of endothelial cells, they were vaguely described as channels without walls, which communicated by open mouths with body cavities and tissue spaces. These channels were thought to be connected with the blood vessels either by hollow connective tissue cells or by hypothetical "vasa serosa," of smaller caliber than capillaries. With the discovery of lymphatic endothelium, tissue spaces came to be considered as the "roots" of the lymph vessels, with whose distal ends tissue spaces were supposedly continuous. It is now known, however, that lymphatic capillaries end blindly and that all lymphatic vessels are separated by their endothelial walls from the tissue spaces.

Investigators of the embryonic origin of lymphatic vessels in birds have adhered to three principal concepts of derivation. First, there is the theory that lymphatic plexuses arise from isolated vascular elements which differentiate in the mesenchyme adjacent to veins and which successively possess, lose, and re-form venous connections during the course of their evolution as lymphatics (*Miller, 1912*). Second, it is said that lymphatics develop through the confluence of intercellular spaces in the mesenchyme which connect secondarily with veins, the lymphatic endothelium being produced by the flattening of the mesenchymal cells that bound the spaces (*West, 1915*). Third, it is contended that all lymphatic vessels arise through the sprouting of the endothelium of veins and grow centrifugally by cell division (*Clark and Clark, 1912, 1920; Sabin, 1916a, 1916b, 1916c*), the lymphatic endothelium showing its specificity immediately upon its origin by its avoidance of the blood capillaries (*Clark and Clark, 1912*). The limitations of various experimental methods probably account for the disparity in these views. The greatest weight of evidence seems to favor the third hypothesis.

### The Lymphatic Vessels

The development of all the lymphatic vessels of the avian embryo has not been investigated. Accounts are available, however, of the early history of the posterior lymph hearts, the jugular lymph sacs (later transformed into lymph glands), the thoracic ducts and large lymph trunks, and the superficial lymph channels of the lateral body wall and pelvis. Studies have been made mainly on the chick embryo.

In general, the growth of lymphatics begins with the elaboration of a deep plexus of lymphatic capillaries from veins. Sprouts from the deep



lymphatic capillaries rapidly spread centrifugally and establish a superficial lymphatic capillary plexus. Lymphatic sacs and ducts then arise through the confluence of capillaries.

### *The Posterior Lymph Hearts*

Stannius (1843) observed the presence of a pair of lymph hearts in the stork (*Ciconia*), the ostrich (*Struthio camelus*), a cassowary (*Casuarius* sp.), a swan (*Cygnus* sp.), a grebe (*Colymbus* sp.), and an auk (*Alca* sp.). In the chicken, lymph hearts exist only during incubation and for a short

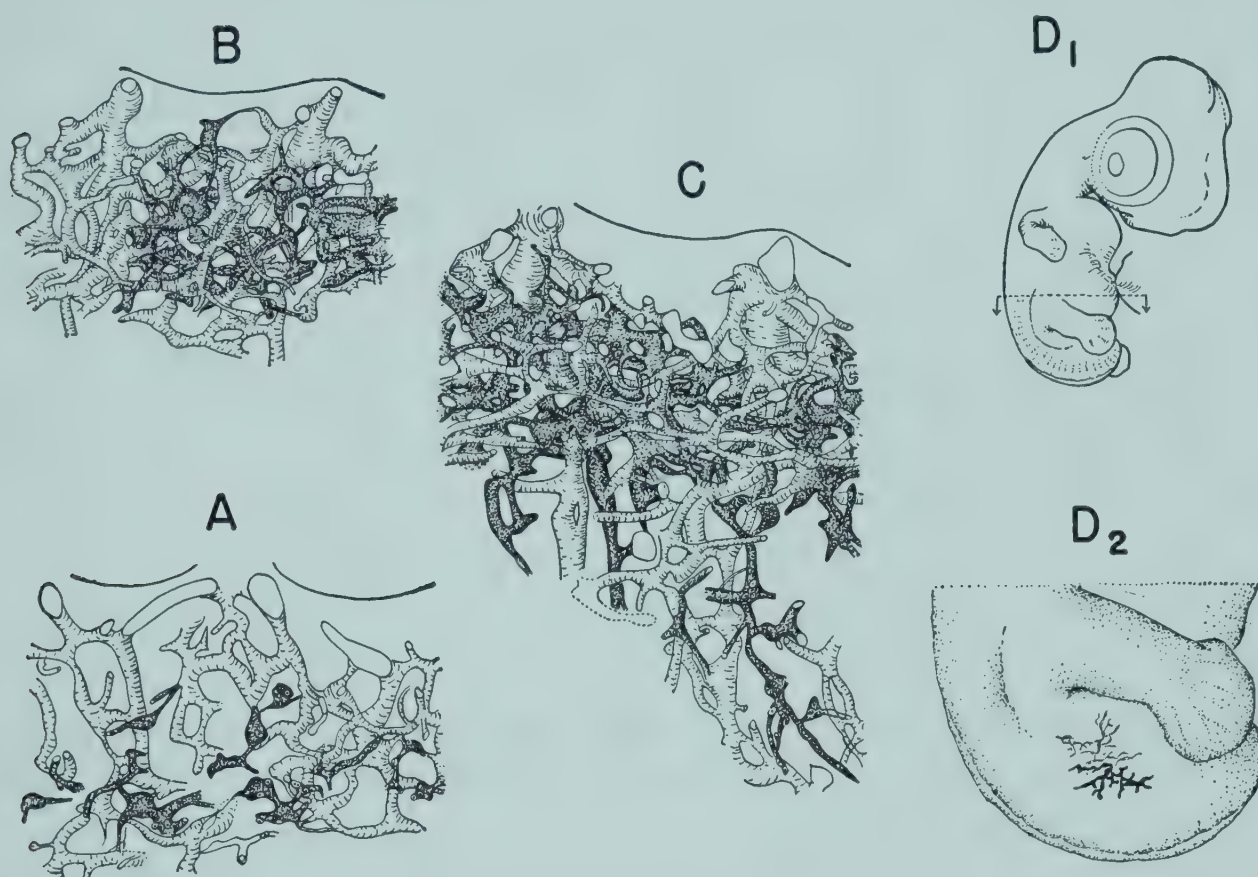


Fig. 262. Increasing complexity of the deep lymphatic plexus (shown as dark vessels) in the region of the posterior lymph heart of the developing chick. (Redrawn with modifications from Clark and Clark, 1920.)

A, the deep lymphatic plexus in the chick embryo of 4 days 7.5 hours ( $\times 70$ ); B, deep lymphatics of the same regions at 4 days 9.5 hours ( $\times 70$ ); C, at 4 days 23 hours ( $\times 70$ ); D<sub>1</sub>, D<sub>2</sub>, at 5 days 12 hours, with the superficial lymphatics shown as lighter vessels ( $\times 5$ ,  $\times 15$ , respectively).

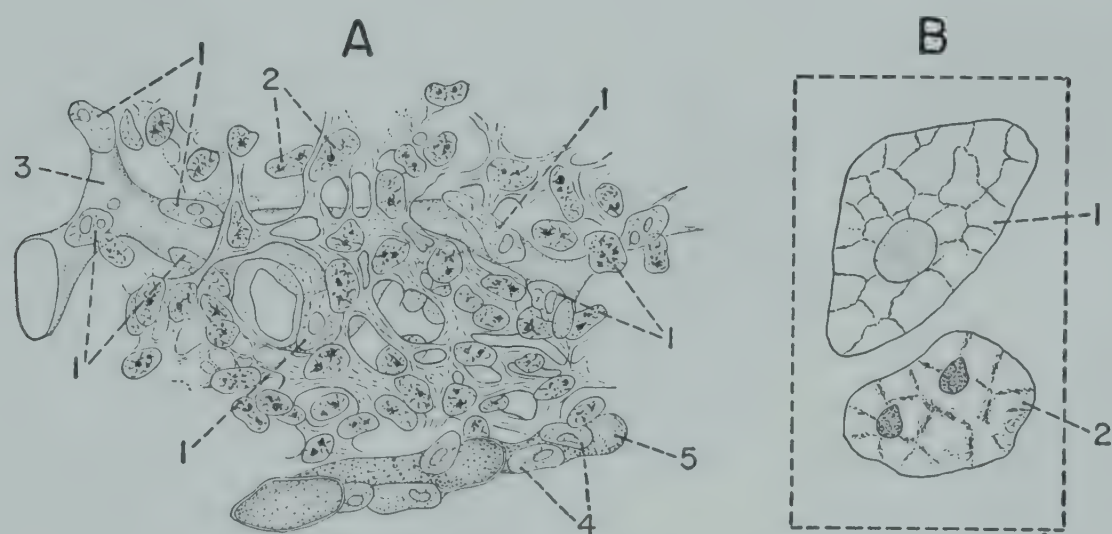
time after hatching. Their embryonic function is thought to be largely concerned with the drainage of lymph from the allantois.

The posterior lymph hearts of the chick embryo were first observed by Budge (1882), and their development has been described by Sala (1900), Mierzejewski (1909), Clark and Clark (1912, 1920), and West (1915). These organs are superficial and lateral to the somites and are at first situated on either side of the tail in the angle between the tail and the posterior border of the pelvis. After the chick's tenth incubation day, they move dorsally and anteriorly as the curve of the vertebral column is reduced (Sala, 1900; Clark and Clark, 1914b).



It is agreed that the primordial lymph hearts are connected with the first five intersegmental coccygeal veins, although it has been said that they originate from spaces in the mesenchyme (*Sala, 1900; Mierzejewski, 1909; West, 1915*); the lymph hearts probably arise through the confluence of endothelial sprouts that grow out from the coccygeal veins and form plexuses (*Clark and Clark, 1912, 1920*).

The earliest trace of the lymphatic plexuses from which the lymph hearts seem to be derived has been found in a chick embryo incubated 4 days 7 hours (*Clark and Clark, 1920*). Figure 262-A, which represents a reconstruction of this embryo, shows that no true plexus of lymphatic capillaries is present at this stage, but that small vessels are sprouting from the endo-



**Fig. 263.** Histological distinctions between mesenchymal and endothelial cells in the developing chick embryo. (Redrawn with modifications after *Clark and Clark, 1920*.)

A, drawing of a portion of the lymph heart region of the 5-day chick sectioned parallel to the surface, stained with Ehrlich's hematoxylin, counterstained with eosin, orange G, and aurantia ( $\times 500$ ); B, nucleus of a lymphatic endothelial cell and of a mesenchymal cell ( $\times 2000$ ).

1, lymphatic nucleus; 2, mesenchymal nucleus; 3, lymphatic; 4, blood vessel nucleus; 5, blood vessel (injected with India ink).

thelium of the venous capillaries. A slightly later stage is shown in Fig. 262-B, reconstructed from an embryo incubated 4 days 9 hours. Here there is a definite lymphatic plexus connected at numerous points with the veins. After incubation for 4 days 23 hours (Fig. 262-C), the plexus is considerably more luxuriant, some of the capillaries are as large as blood capillaries and contain blood cells, some are quite narrow, and some consist of solid endothelial processes. As Fig. 263-A and B show, lymphatic endothelium, even at this early stage, is distinguishable from mesenchymal tissues by its nuclear structure (*Clark, 1914; Clark and Clark, 1920*).

In the living 5-day embryo, the early lymphatic plexus in the lymph heart region appears as clusters of blood-filled "knobs" attached to the veins. The contained blood is stagnant and in all probability has been



forced into the early lymphatics by back-pressure from the veins (*Clark, 1912a; Clark and Clark, 1912*), although an alternative hypothesis suggests that red blood cells differentiate *in situ* in this region and are enclosed within the lymphatics as the latter develop (*West, 1915*).

In 5- to 5.5-day chick embryos (cf. Fig. 262- $D_1$  and  $D_2$ ), the blood-filled deep plexus has given rise to a superficial plexus, which lies, however, beneath the superficial blood capillaries. In embryos a day older, the superficial plexus has spread out over the pelvis and has become part of a superficial lymphatic plexus that is continuous from the tail to the axilla, where it connects with another deep lymphatic plexus (the jugular plexus) lying dorsal to the anterior and posterior cardinal veins near their point of juncture (*Mierzejewski, 1909; Clark and Clark, 1920*).

By the time the 8-day stage is reached, the lymph hearts have been transformed from plexuses to chambered oval or almond-shaped sacs (*Budge, 1882; Sala, 1900; E.L. Clark, 1915a; Clark and Clark, 1914b, 1920*). *Sala (1900)*, who did not observe the anlagen of the lymph hearts prior to the 6.5-day stage, stated that mesenchymal trabeculae from the walls of each sac grew into its lumen at the 7.5-day stage. It is more likely, however, that the trabeculae seen by *Sala* represent the walls of greatly enlarged channels formed by the coalescence of capillaries. The expansion of the small channels of the lymph heart is probably due to the rapid passage of fluid through it from the allantoic lymphatics, which, at the 8-day stage, have attained considerable length (*E.L. Clark, 1915a*). The large cavities or channels within the saclike lymph heart continue to coalesce during the ninth and tenth days, and the trabeculae have disappeared by the fifteenth day (*Sala, 1900*).

Each posterior lymph heart may have lost its connections with two of the coccygeal veins by the 8-day stage (*E.L. Clark, 1915a*), although *Sala (1900)* observed that this reduction in venous connections may take place at various times between the ninth and sixteenth days and usually occurs on the eleventh or twelfth day. He stated that it is the first and fifth veins that cease to communicate with the lymph heart.

Valves form between the lymph hearts and the veins at the 7.5- to 8-day stage, and blood no longer enters the lymphatics from the veins. After the ninth day, a valve forms between the lymph heart and the superficial lymphatics (*E.L. Clark, 1915a*).

According to *Sala (1900)* and *Mierzejewski (1909)*, muscle fibers begin to appear in the mesenchymal connective tissue forming the wall of the lymph heart in the 9-day chick embryo; but *Clark and Clark (1914b)* observed muscle fibrillae around the plexiform lymph heart at the 6.5-day stage, at the time when the lymph heart begins to contract. The myotomes may perhaps be the source of these fibrillae (*Clark and Clark, 1914b*). The muscle fibers form a layer in direct contact with the endo-



thelial lining of the heart, but, by the 13-day stage, a layer of connective tissue intervenes between the two (*Sala, 1900*).

In the opinion of E.L. Clark (*1915a*), the development of the muscular layer around the lymph hearts prevents their subsequent expansion, but the data of Sala (*1900*) do not bear out this viewpoint. The latter author reported that the maximum diameter of each lymph heart cavity is 0.2 mm. at the end of the seventh day and that it increases to 0.7 to 0.9 mm. by the tenth day and to 1.5 to 2.0 mm. by the fourteenth or fifteenth day, after which time it remains unchanged. He found the thickness of the wall to be 0.035 mm. on the tenth day, 0.05 mm. on the fifteenth day, and 0.125 mm. (maximum 0.2 mm.) at hatching.

The investigations of Clark and Clark (*1914a, 1914b*) have shown that the first contractions of the lymph hearts, occurring in the 6.5- to 7-day chick embryo, are always associated with the normal spasmodic movements of the tail. The lymph hearts beat several times during each body spasm. If the body movements are prevented by the application of chloretone, lymph heart contractions do not take place. The lymph hearts may be stimulated to beat by puncture of their walls, a procedure which, at this stage, also evokes movements of the tail.

Subsequently, the lymph hearts gradually become capable of functioning independently. At the 7- to 7.5-day stage, the hearts sometimes contract in the intervals between body movements; at the 8-day stage, they beat about five times during each spasm, occasionally during the intervals between spasms, and four or five times per minute when the body movements are paralyzed by chloretone. A day later, three or four pulsations of the lymph hearts occur during each periodic body movement and also during each interval of rest. In embryos incubated 10 to 13 days, the lymph heart contractions have become entirely independent of the periodic body spasms and occur from four to eight times per minute.

Clark and Clark (*1914b*) were unable to discover a definite anatomical relationship to account for the close functional association that exists at first between the body musculature and the lymph hearts. No direct connection between the contracting myotomes and the muscle fibrillae of the lymph hearts could be demonstrated, although the walls of the lymph hearts are close to the lateral portions of the myotomes in the 6.5- to 7-day embryo. Purely mechanical stimulation is unlikely, because the dissociation of lymph heart function from body contractions is gradual, whereas the isolation of the lymph hearts in the mesenchyme at the 8- or 9-day stage occurs suddenly when the lateral portions of the myotomes disappear.

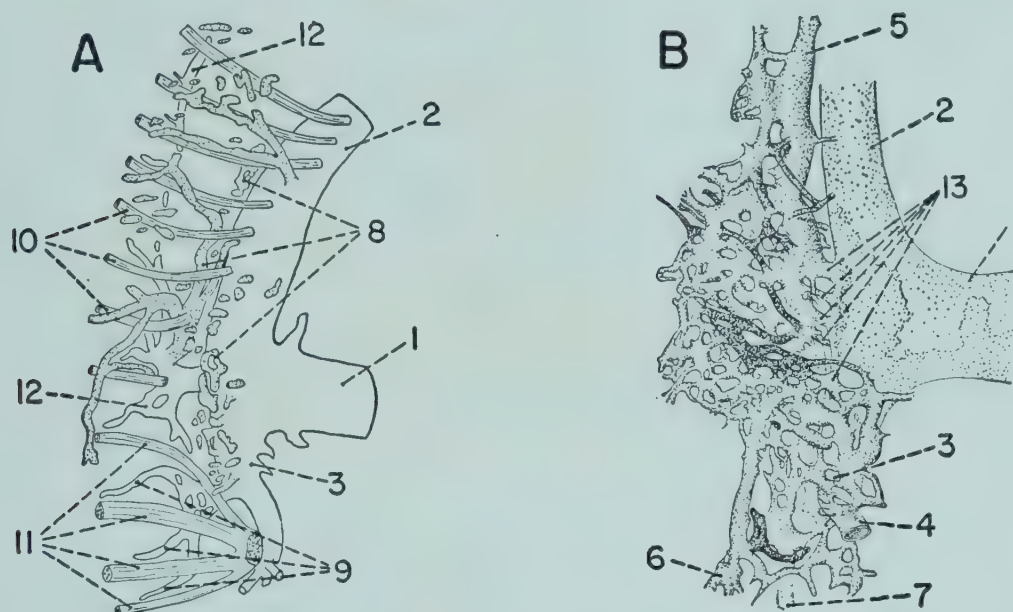
It is possible that the increasing independence of the lymph heart pulsations is due to the growing intensity of the internal stimulus provided by



the ever larger flow of lymph. It has been shown that the lymph hearts react to an internal stimulus, such as injected fluid (*Clark and Clark, 1914b*).

### *The Jugular Lymphatic Plexuses*

The main lymphatic vessels in the neck region of birds consist of paired trunks which become continuous caudally, at the level of the superior venae cavae, with the thoracic ducts (*Sala, 1900*). In a few birds, notably the palmipeds (*Jolly, 1910*), a pair of lymph glands is found at the point of transition between the cervical (jugular) trunks and the thoracic ducts. These lymph glands are derived from a luxuriant plexus, the deep jugular



**Fig. 264.** The jugular lymphatic plexus of the chick embryo during the sixth day of development. (Redrawn with modifications A, after *Miller, 1912*; B, after *Clark, 1912b*.)

A, a diagrammatic reconstruction of the jugular lymphatic plexus of the chick embryo of 5 days 10 hours of incubation; B, the same plexus in a slightly older chick (5 days 20 hours) as shown by injection ( $\times 25$ ).

1, duct of Cuvier; 2, anterior cardinal vein; 3, posterior cardinal vein; 4, thora-coepigastric vein; 5, cervical lymphatic duct; 6, vessel from the superficial lymphatic plexus; 7, deep lymphatic; 8, isolated lymph islands and channels; 9, intersegmental veins; 10, cervical spinal nerves; 11, brachial plexus; 12, vertebral vein; 13, vessels connecting jugular lymphatic plexus with veins.

lymphatic plexus, which appears during embryonic development even in those species (such as the chicken) which do not possess cervical lymph glands in adult life.

The deep jugular lymphatic plexus begins to develop in the second half of the chick embryo's sixth incubation day (*Mierzejewski, 1909; Miller, 1913*) and very soon elaborates a superficial plexus in the axillary region (*Mierzejewski, 1909*). According to *Mierzejewski (1909)*, the lymphatic capillaries of the deep plexus originate as small branches of the jugular, subclavian, and vertebral veins. *Miller (1913)* described a more complex



derivation from "venolymphatics," or isolated vascular islands and channels appearing in the mesenchyme and connected here and there with the anterior and posterior cardinal veins and the intersegmental veins. The increase in the number and complexity of the venolymphatic elements in the 6-day embryo is accompanied by the loss of all connections with the veins. At the 7-day stage, the plexus becomes a sac which, in the 8.5-day embryo, forms a secondary connection with the jugular (anterior cardinal) vein.

Miller's (1913) version of the development of the jugular lymphatic plexus was based on the study of serial sections and was questioned by Clark (1912*b*), who injected the lymphatics with India ink. The injected deep jugular plexus of the 6-day embryo may be seen in Fig. 264-B, which is to be compared with Fig. 264-A, representing the plexus at the same developmental stage but reconstructed from sections. In the injected specimen, at least five connections with the anterior cardinal vein are visible. Clark (1912*b*) found that the number of connections is reduced to two by the eighth incubation day. She was unable to corroborate the early transformation of the plexus into a sac and concluded that the sac reported by Miller (1913) was merely the largest vessel of the plexus.

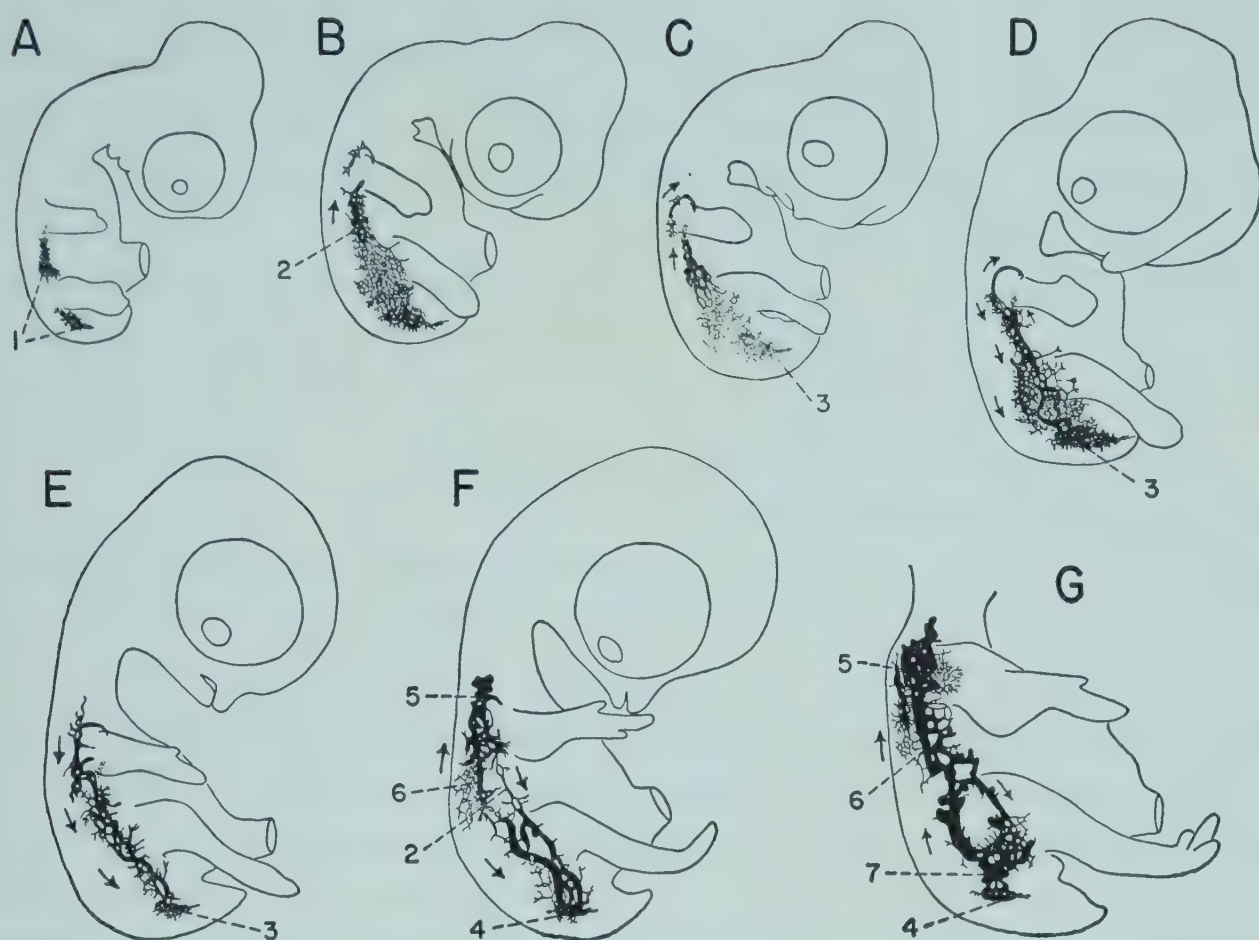
#### *The Superficial Lymphatics of the Body Wall*

The development of superficial lymphatics in the lateral body wall of the chick embryo has been described by Mierzejewski (1909) and E.L. Clark (1915*a*, 1915*b*). Their accounts are in substantial agreement, with the exception that there is a temporal difference of 24 to 36 hours between the two reports, the earlier author's embryos apparently being retarded in development.

In the 5- or 5.5-day embryo, there are two superficial lymphatic plexuses. One, situated over the pelvis and in the tail region lateral to the myotomes, is derived from the deep plexus of the posterior lymph heart; the other, derived from the deep jugular plexus, extends from the axilla to the leg in the lateral body wall over the thoracoepigastric vein (Fig. 265-A). These plexuses are both filled with stagnant blood. In embryos about 6.5 days old (cf. Fig. 265-C), a distinct channel has formed in the lateral plexus, which has now become continuous posteriorly with the pelvic plexus. Lymph is flowing cephalad through this channel to the axilla, where it proceeds medially to the deep jugular plexus. There is no movement of lymph posterior to the leg, however.

The next lymphatic capillaries to appear superficially arise from the deep jugular plexus, emerge in front of the shoulder, and grow around to the dorsal surface of the shoulder. In 6.5-day embryos, in which the posterior lymph heart is beating, the lymph flows anteriorly in the lateral channel and in a channel that has differentiated in the newly formed





**Fig. 265.** Successive stages in the development of superficial lymphatic vessels within primitive lymphatic plexuses in the developing chick embryo. (Redrawn with modifications after E. L. Clark, 1915a.)

A, injected embryo of 5 days 12 hours showing the primary anterior and posterior lymphatic plexuses; B, injected chick of 5 days 22 hours (the stage of beginning lymph flow), showing first lateral lymphatic differentiating in the side plexus and extension of the remainder of the superficial lymphatics, which retain their primitive character; C, injected chick of 6 days 12 hours (the stage of beginning lymph heart pulsations, earliest posterior circulation over the pelvis, and beginning anterior flow of lymph in the suprascapular plexus); first posterior channels and suprascapular channel are shown differentiating in the injected primary plexuses; D, injected chick of 6 days 22 hours (the stage of stronger lymph heart pulsations and posterior circulation in most of the superficial lymphatics), showing newly formed channel connecting suprascapular lymphatics with those of the side, and large channels over the side and pelvis; E, injected chick of 7 days (the stage of rapid posterior circulation into the lymph heart from all of the superficial lymphatics), showing large, comparatively straight channels over the surface of the body and enlarged channels through the lymph heart; F, injected chick of 8 days (the stage of beginning anterior circulation in the dorsal part of the side lymphatics and retarded flow in the suprascapular region), showing saccular lymph heart and main lymph channels; G, injected chick of 9 days (the stage of anterior circulation for side region and part of the pelvis, posterior circulation for the rest of the pelvis, and retarded flow in the suprascapular region and over the posterior surface of the pelvis), showing a new lateral lymph channel which has replaced the original ventral one. All  $\times 2$ .

Arrows indicate path and direction of circulation in the living embryo. 1, superficial lymphatic plexus; 2, first lateral lymphatic; 3, lymph heart (plexus); 4, lymph heart (sac); 5, suprascapular lymph sac; 6, newly formed lateral lymph duct; 7, pelvic lymph sac.



suprascapular plexus (cf. Fig. 265-C). Channels have also appeared in the midst of the lymphatic plexus of the pelvis. The flow of lymph has now begun in this region, but it is directed posteriorly, toward the lymph heart. By this time the moving lymph has forced the stagnant blood out of all the superficial lymphatics.

By the end of the seventh day, the flow of lymph in the superficial lymphatics is directed posteriorly except in the axilla and in the most anterior part of the suprascapular channel. Much larger channels are now present in all regions (cf. Fig. 265-D). A few hours later, no anterior flow of lymph is seen anywhere (cf. Fig. 265-E).

At the 7.5-day stage, a new superficial plexus of capillaries grows posteriorly from the suprascapular plexus. These capillaries are situated parallel and dorsal to the lateral channel that is already present. With their appearance, the flow of lymph in the lymphatics dorsal to the shoulder is again reversed and once more proceeds anteriorly to the deep jugular plexus. The original lateral channel gradually disappears after 8 days of incubation. In the 8-day embryo, the development of the shoulder cartilage has interfered with the free flow of lymph into the deep jugular plexus, and a large lymph sac starts to develop in the suprascapular region (cf. Fig. 265-F).

In the embryo incubated 8.5 to 9 days (cf. Fig. 265-G), the suprascapular sac is much larger, probably because the pressure of the lymph in it is increased as lymph flow into the jugular sac is retarded by greater lymphatic drainage from the deep lymphatics of the neck and lungs. A similar sac has now appeared close to the posterior lymph heart. The large posterior lymph channels have separated into dorsal and ventral vessels, and the posterior lymph sac lies at the point of juncture between them. The lymph, flowing posteriorly in the ventral channel, pauses in the sac and enters the lymph heart if a pulsation of the latter occurs; if the lymph heart does not contract, the lymph flows anteriorly through the dorsal channel and eventually finds its way to the jugular sac. The posterior sac, therefore, forms at the site of retardation in superficial lymph flow, the retardation in turn being caused by the increased drainage of lymph into the lymph heart from the allantois.

By various operative procedures, Clark and Clark (1920) demonstrated that the superficial lymphatics of both the tail region and the lateral body wall can and probably do originate from other sources than the jugular plexus and the lymph heart plexus. Development *in situ* can also occur.

#### *The Thoracic Ducts and the Para-Aortic Lymphatic Trunks*

The paired thoracic ducts of birds course parallel and ventrolateral to the aorta and the aortic roots in the thoracic region and empty into the superior venae cavae medial and proximal to the jugular veins. Although



directly continuous posteriorly with a similar pair of large para-aortic lymphatic trunks extending to the caudal extremity of the body, the thoracic ducts are usually considered as terminating at the level of the coeliac artery, where a lymphatic plexus more or less interrupts the cephalocaudal continuity of the system of paired lymphatic vessels. This distinction between the thoracic ducts and the two lower lymphatic trunks is further justified by the observation that the latter develop in the embryo from posterior to anterior and do not make juncture with the former until about midway through the incubation period (*Sala, 1900*).

The thoracic ducts, before debouching into the venae cavae, are often joined by the lymphatics that drain lymph from the head, neck, lungs, and wings, although these lymphatics may have separate mouths. Into the plexus around the aorta at the root of the coeliac artery empty the lymphatics accompanying this artery and its rami; posterior to the coeliac artery, the paired lymphatic trunks receive lateral rami that collect lymph from the entire caudal half of the body, including the legs (*Hewson, 1768; Lauth, 1824; Sala, 1900*).

Unfortunately, there exist very few descriptions of the development of the thoracic ducts in avian embryos. The most detailed accounts, those of *Sala (1900)* and *Miller (1913)*, provide some information on the chronological morphogenesis of the ducts in the chick but are open to criticism from proponents of the theory that lymphatics originate from venous endothelium. *Sala (1900)* stated that the thoracic ducts (as well as the lower lymphatic trunks) are formed by the cavitation of solid strands of mesenchymal cells intermingled with red blood cells. It is to be noted, however, that he found this process of cavitation beginning in 8-day embryos, whereas he had already observed numerous endothelium-lined spaces around the thoracic aorta in embryos incubated 7 days 6 hours. These spaces he interpreted as lymph channels corresponding to the "primary lymph circulation" of *Budge (1881, 1887)*. The latter author proposed that the lymphatic vessels of the chick embryo first formed a primary closed system of canals which did not communicate with the blood vessels and which was superseded by a secondary system connected with the blood vessels by means of the lymph hearts and thoracic ducts. It seems implicit in *Sala's* report that this investigator did not observe the earliest stages of thoracic duct development.

According to *Miller (1913)*, red blood cells differentiate from mesenchymal cells in the vicinity of the aorta of the 5- and 6-day embryo. In the 6.5- to 7-day embryo, the anlagen of the thoracic ducts form when intercellular spaces in the mesenchyme begin to coalesce into larger spaces. The mesenchymal cells lining the spaces gradually differentiate as endothelial cells. Some of the red blood cells are enclosed within the forming lymphatic spaces and others migrate into them, so that the thoracic ducts



perform a "hemophoric" function by (eventually) carrying these red blood cells into the blood circulatory system (*Huntington, 1914*). This hemophoric function of lymphatic vessels has been denied by Clark and Clark (1920) on the grounds that all the blood contained in lymphatic vessels is stagnant blood forced in from the veins with which the lymphatics are connected. Sala's solid anlagen, therefore, may perhaps have been lymphatic vessels filled with blood.

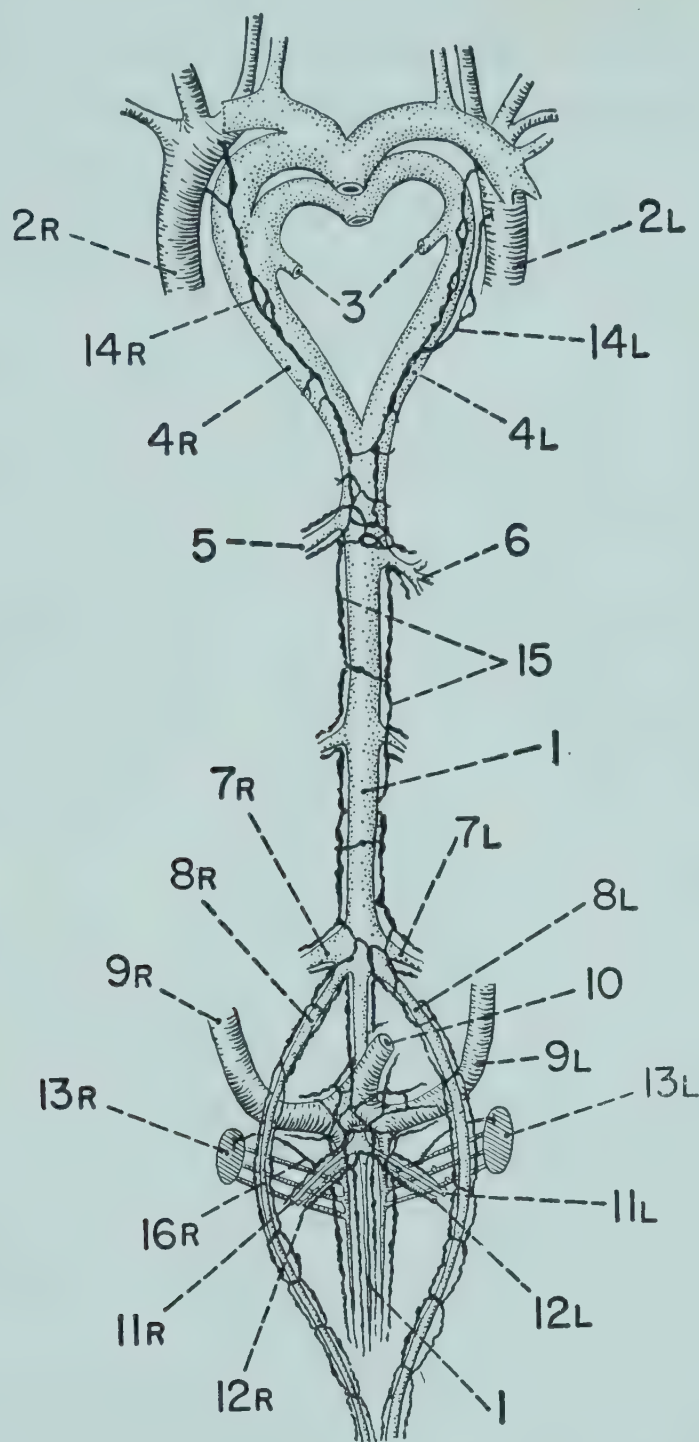
Both Sala (1900) and Miller (1913) described the early thoracic ducts as being discontinuous and stated that the ducts grow and become continuous by the addition of newly differentiated anlagen (intercellular spaces or cellular aggregates). Neither author mentioned any communication with blood vessels during the first days of development. It is possible, however, that injection of India ink, rather than examination of sections, might indicate that the thoracic ducts are formed in the chick, as they have been said to form in mammals, by the downgrowth of a plexus from the jugular lymphatic plexus. In Fig. 264-B, which represents the injected jugular plexus of the 6-day chick embryo, the jugular plexus is shown as continuing posteriorly with a deep lymphatic plexus (*Clark, 1912b*). Sala (1900), in fact, indicated that there is a continuity between the anlagen of the jugular lymphatics and the thoracic ducts and stated that the development of the jugular vessels advances more rapidly than that of the thoracic vessels.

As to the anteroposterior growth of the thoracic ducts, Sala (1900) found their anlagen extending as far as the confluence of the dorsal aortic roots in the 7.5-day embryo and down to the root of the coeliac artery in the 8-day embryo. Miller (1913), however, observed a few isolated rudiments caudal to the root of the coeliac artery in an embryo incubated 6 days 16 hours. The latter author also noted the presence of channels growing out from the jugular lymph sacs (or plexuses) toward the thoracic duct anlagen of an embryo only 5 hours older, and stated that these channels connect the jugular sacs and the thoracic ducts in the 8-day embryo. In the 9-day embryo, he found additional connections between the jugular sacs and the thoracic ducts in the form of continuous lymphatic vessels situated dorsolateral to the aorta and derived from anlagen already present of the 7-day stage.

The developmental history of the posterior lymphatic trunks, as given by Sala (1900), begins with the 9-day stage. At this time, there are no anlagen of the lymphatic trunks between the level of the coeliac artery and the kidneys. More posteriorly, there are endothelial-lined spaces that tend to coalesce into two larger canals running parallel and dorsolateral to the aorta and extending even into the tail. There also exists a lymphatic plexus around the roots of the umbilical and ischiadic arteries. In the embryo incubated 10 days 17 hours, Sala found the two trunks reaching



somewhat farther anteriorly, and noted that they become continuous with the thoracic ducts in the 12-day embryo. It should be noted, however, that an anteroposterior continuity of all the deep lymphatics around the aorta was observed by E.L. Clark (1915b) in the 9-day embryo. Sala also re-



*Fig. 266.* Schematic figure of main lymph channels in the 17-day chick embryo, showing their close relationship to the blood vessels. (Redrawn after Sala, 1900.)

1, aorta; 2, superior vena cava; 3, pulmonary artery; 4, root of aorta; 5, coeliac artery; 6, mesenteric artery; 7, ischiadic artery; 8, umbilical artery; 9, hypogastric vein; 10, coccygeo-mesenteric vein; 11, pudendal artery; 12, pudendal vein; 13, posterior lymph heart; 14, thoracic duct; 15, lymphatic trunk; 16, lymphatic vessel from lymph heart. (L, left; R, right.)

ported the existence of a lymphatic plexus (the “cruciate” plexus) around the junction of the coccygeo-mesenteric and hypogastric veins at the 12-day stage. His diagram of the thoracic and abdominal lymphatics of the 16- to 17-day embryo is reproduced in Fig. 266. In the chick of this age, there is an abundant lymphatic plexus dorsal and lateral to the bursa of Fabricius



and the cloaca and another around the pudendal arteries and veins; the latter plexus receives direct branches from the lymph hearts.

### The Lymph Glands

Lymph glands are found in only a few birds, and their number is usually limited to two pairs. These are the cervical glands, derived from the jugular lymph sac, and the lumbar glands, situated along the course of the para-aortic lymphatic trunks between the ischiadic and femoral arteries. The lumbar glands are considerably larger than the cervical glands. A third pair of "thoracic" glands, near the cervical glands, has been seen in the goose, *Anser anser* (Lauth, 1824) and the swan, *Cygnus* sp. (Jolly, 1910).

Lymph glands seem to be confined to certain species of aquatic and wading birds. They were first observed in the goose (*Anser anser*), in which the cervical glands were discovered by Hewson (1768) and the lumbar glands by Pensa (1907), although Panizza (1830) apparently knew of the latter glands. Jolly (1910), who examined members of several orders of birds, found lymph glands only in certain *Anseriformes*—specifically, the mute swan (*Cygnus olor*), the black swan (*Chenopsis atrata*), the domestic goose (*Anser anser*), domestic and wild ducks (*Anas platyrhynchos*, *Cairina moschata*, *Anas acuta*), a sheldrake (*Casarca tador-noides*), and two teals (*Anas querquedula*, *Anas crecca*).

Structurally, the lymph glands of birds differ from those of mammals in that the principal sinus, representing the continuation of a lymphatic channel, is located centrally rather than peripherally. In addition, the relative position of the lymphoid and the spongy or reticular tissue is reversed; the lymphoid tissue, containing the nodules or lymphopoietic centers, is medial to the spongy tissue and surrounds the central sinus. Lastly, the blood vessels do not enter through a single hilus but penetrate the cords of the reticular tissue at many points.

The embryonic development of avian lymph glands has been observed by Pensa (1907) and by Jolly (1910), who studied the goose (*Anser anser*) and the duck (*Anas platyrhynchos*), respectively. The reports of these two investigators are more or less in accord, although Jolly questioned the seeming precocity of development in the embryos examined by Pensa.

In the duck, the cervical and lumbar glands develop identically and practically simultaneously. The initial stage in their evolution is seen at the 12-day stage, when very small buds of mesenchymatous tissue project slightly into the cervical and lumbar enlargements of the respective lymphatic ducts. The endothelium is displaced inward by the tissue buds. In the period between the fourteenth and eighteenth days, many of the buds meet and fuse, forming septa that span the lumen (Fig. 267-A). These septa represent the future cords of the reticular tissue, and the spaces between them represent the future sinuses (which will be connected with the



central sinus by intermediary sinuses passing through the lymphoid tissue). By the twenty-second day (cf. Fig. 267-B), each cervical gland has the aspect of a small spongy mass with a central sinus. Blood vessels from the neighboring connective tissue have penetrated into the principal cords of the developing reticular tissue. Also, the first lymphoid cells have appeared and are beginning to accumulate in the cords around the central sinus. This stage of development is reached about 4 days later in the lumbar glands than in the cervical glands.

After 26 days' incubation (cf. Fig. 267-C), the lymphoid tissue of the cervical glands has increased in amount. Mitotic activity is widespread

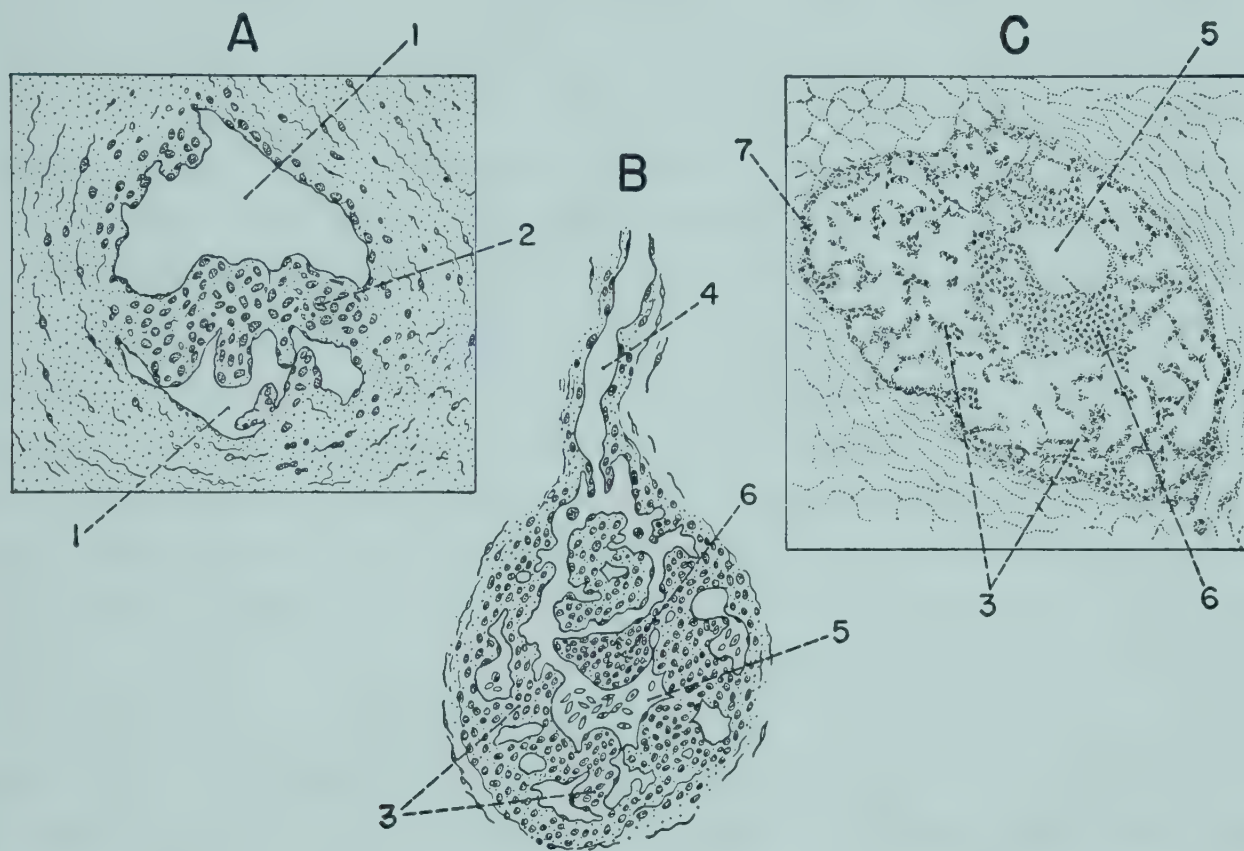


Fig. 267. The development of the cervical lymph glands in the embryo of the duck, *Anas platyrhynchos*. (Redrawn with modifications after Jolly, 1910.)

A, 18-day stage ( $\times 180$ ); B, 22-day stage ( $\times 120$ ); C, 26-day stage ( $\times 60$ ).

1, lumen of lymphatic vessel; 2, septum formed of fused mesenchymatous buds; 3, reticular tissue of gland; 4, afferent lymphatic vessel; 5, central sinus of gland; 6, early lymphoid tissue; 7, capsule of gland.

throughout this tissue, rather than being confined to the lymphopoietic centers (nodules). The latter do not appear in either the cervical or lumbar glands until well after hatching, and they are still few in number in the 2-months-old duck (*Anas platyrhynchos*).

## THE SPLEEN

The spleen is a small organ situated dorsal to and somewhat to the right of the proventriculus. Depending upon the species of bird, it may be round, oval, or elongated in shape. It consists of lymphatic tissue of two types, known as the red pulp and the white pulp. The spongy red pulp is



composed of cords of mingled reticular cells and blood elements ramifying between large venous sinuses which communicate with veins. The white pulp is found around small arterial vessels and contains large concentrations of lymphocytes among reticular cells. The chief functions of the spleen are the manufacture of white blood cells and the destruction of red blood cells.

The spleen develops in the left half of the gastroduodenal part of the dorsal mesentery, almost directly dorsal to the dorsal pancreatic primordium. In the chick, it appears during the second half of the fourth day of incubation (*Tonkoff, 1900a; Pinto, 1904; Danchakoff, 1916*); in the duck (*Anas platyrhynchos*), during the first half of the fifth day (*Tonkoff, 1900a*); and in the sparrow (*Passer domesticus*), at the end of the third day or the beginning of the fourth day (*Woit, 1897*).

The spleen is of mesodermal origin, and its first visible anlage is a condensation of cells in the mesenchyme of the dorsal mesentery, close beneath the thickened mesothelium (coelomic epithelium). The mesothelium has been thought to give rise to the cellular condensation both directly, by proliferating cells into the mesenchyme (*Choronshitzky, 1900; Giannelli, 1909*), and indirectly, by proliferating, at an earlier stage, some of the mesenchymal cells that in turn multiply to form the condensation (*Tonkoff, 1900a; Pinto, 1904*). On the other hand, any participation of the coelomic epithelium in the formation of the splenic primordium has been denied (*Danchakoff, 1916*).

Early in the chick's fifth day of incubation, the spleen, about 0.0025 mm. thick, begins to bulge into the body cavity; it extends craniad into the mesogastrium and caudad to the omphalomesenteric vein (*Pinto, 1904*) and may range in length from 0.23 mm. (*Giannelli, 1909*) to 0.4 mm. (*Pinto, 1904*). A corresponding stage is found in the duck (*Anas platyrhynchos*) at the end of the fifth day (*Tonkoff, 1900a*). As its development continues, the spleen becomes thicker and bulges farther into the coelom. On the ninth day, when it is about 1.0 or 1.5 mm. long (*Danchakoff, 1916*), it is a reddish, spherical organ (*Rioch, 1923*). Its color becomes brownish red by the twelfth day; thereafter, the only gross change is its growth to a length of about 4 mm. by the twentieth day (*Rioch, 1923*).

The cells of the primitive mesenchymal condensation undergo intense multiplication and have formed a syncytium by the chick's sixth or eighth day. Almost from the beginning, the spleen contains blood vessels stemming from the omphalomesenteric vein (*Tonkoff, 1900a; Pinto, 1904; Giannelli, 1909*). By the eighth day, the spleen (now covered by a single layer of flat peritoneal cells) starts to acquire the spongy structure characteristic of the red pulp (*Giannelli, 1909; Rioch, 1923*). Beginning at the periphery of the organ, clefts appear among the cells, and these clefts soon join to form a network of spaces which become venous sinuses when



the network communicates with the veins. The mesenchymal cells bordering the sinuses flatten and give the sinuses a smooth internal surface. Meantime, other mesenchymal cells have begun their differentiation as lymphoid hemocytoblasts or (when included in the venous sinuses) as erythroblasts. On the twelfth or thirteenth day (by which time granulocytopoiesis has begun in the pulp), arterial vessels start to appear in the spleen; these grow in from outside and ramify in the pulp by extensive budding. The mesenchymal cells proliferate intensely around the arteries and their small rami, until, by the fifteenth day, small islands of mesenchymal cells are formed around the arterial vessels; these islands constitute the primordial white pulp. Between the fifteenth and the seventeenth day, when small lymphocytes begin to be formed in the white pulp, the connections between the venous and the arterial vessels are complete (*Danchakoff, 1916*).

The embryonic chick's spleen becomes greatly enlarged if a fragment of adult chick spleen is implanted on the chorioallantois on the sixth to tenth day of incubation and grown as a graft until the seventeenth or eighteenth day (*Danchakoff, 1916; Murphy, 1916; Sandstrom, 1932; Pomerat, 1949; Ebert, 1951*). Certain other adult tissues, especially liver (*Murphy, 1916; Willier, 1924*), thyroid, thymus (*Willier, 1924*), bone marrow, and kidney (*Murphy, 1916*), have a similar but much less marked effect. The hypertrophy is traceable to a stimulated production of white blood cells (*Danchakoff, 1916; Murphy, 1916; Willier, 1924; Sandstrom, 1932*). *Danchakoff (1916)* noted that the reaction of the embryonic spleen to the presence of an adult spleen graft varies somewhat with age at the time the graft is implanted. If the stimulation of adult splenic tissue is applied before the eighth day, while the spleen is still syncytial and not well vascularized, there is such an intense cellular proliferation and granuloblastic differentiation as to convert most of the splenic anlage into free amoeboid cells. Development of the sinuses is defective, and the consequent accumulation of granulocytes leads eventually to necrosis. Arterial vascularization is also poor, and the mesenchymal cells, in regions lacking a proper blood supply, proliferate as fibroblasts, so that large parts of the spleen are transformed into connective tissue. Stimulation applied on the ninth day, when the formation of sinuses is active, has the additional effect of temporarily accentuating erythropoiesis; if it is applied after the twelfth day, the production of lymphoid hemocytoblasts at the expense of reticulum cells is intensified throughout the entire organ, and the hemocytoblasts then differentiate as granulocytes or lymphocytes according to their localization in either the red or the white pulp.

Like the presence of anti-spleen serums in the egg (*Pomerat, 1949*), grafts of spleens from chick embryos incubated less than 14 days cause no enlargement of the host embryo's spleen; the capacity of the grafted spleen to stimulate the host's spleen is acquired when the donor's age is 14 to 20



incubation days, and it becomes continually more marked as the donor's age increases to 7 weeks post-hatching. These observations, as well as certain immunological tests, suggest that a specific group of spleen antigens begins to differentiate during the last week of embryonic development (*Ebert, 1951*).

In cultures of the chick embryo's spleen, two types of cells grow out from the explant—the mesenchymal and mesothelial cells, and the free migratory cells, that is, blood elements and macrophages. The mesenchymal and mesothelial cells are the most numerous in cultures from embryos less than 9 days old, and the blood cells are the most numerous in spleen cultures from older embryos (*Rioch, 1923*). In spleen cultures derived from 18-day embryos, the first cells to appear are granular myelocytes, which are followed by lymphocytes and monocytes; fibroblasts appear by the end of the first day of culture, growing out radially (*Rioch, 1923; Pomerat, Jacobson, and Orr, 1949*). Endothelial cells (scarce in the spleen) do not appear in cultures, at least in a medium consisting of Locke-Lewis solution (*Rioch, 1923*).



## CHAPTER NINE

# *The Heart*

*The heart, a center of attraction,  
Has domination of the whole;  
Its bulging muscles are in action  
From early start till final toll.*







# THE HEART

The heart is the physiological center of the vascular system. It pumps blood from a very early period in development until the moment of death. Although the heart can be considered a specialized blood vessel, its structure and functional activity set it apart from the arteries and veins soon after it makes its appearance. Its walls become greatly thickened and preponderantly muscular, and its endothelial lining proliferates structures that divide it into the four-chambered organ characteristic of birds as well as mammals. Its muscular tissue is unique in that it is striated yet apparently requires no external stimulus to initiate or maintain its activity. The spontaneous onset of cardiac function early in embryonic life attests to the vital and dynamic role of the heart.

## THE MORPHOLOGICAL DEVELOPMENT OF THE HEART

The heart begins its development at an early stage, simultaneously with the extraembryonic and intraembryonic blood vessels. Throughout the entire incubation period, it is the connecting link between these two vascular systems.

In the beginning, the heart is a paired tubular structure, formed by the approximation of bilateral primordia, and continuous posteriorly with the omphalomesenteric veins, which empty into it. The double heart is soon replaced by a single tube, and the subsequent course of cardiac development is determined to a great extent by two forces acting upon this tube. One of these is the limitation imposed upon the growing heart by lack of space in which to elongate. The other is the impact of two blood streams upon the inner surface of the already contorted cardiac tube. These influences are largely responsible for the external configuration of the heart and for the development of all the internal structures which eventually divide its cavity into right and left halves each consisting of two chambers, an auricle and a ventricle.

### The Cardiogenic Material of the Early Blastoderm

The cells in the chicken blastoderm that are destined to take part in heart formation are determined not only before the first recognizable primordia of the heart appear but even before the egg is laid. The early origin



of specific cardiogenic potency is indicated by the differentiation of cardiac tissue in unincubated, primitive streak, and head-process blastoderms explanted to culture media or chorioallantoic membranes (*Olivo, 1928b, 1928e, 1928f; Willier and Rawles, 1931a, 1931b; Butler, 1935*).

The distribution of prospective heart material in the blastoderm appears to be influenced by the morphogenetic movements that occur during the early stages of development (see Chapter 3). The location of cardiogenic cells before incubation has not been definitely established, but there is evidence that they are probably most numerous in the peripheral (*Olivo, 1928b, 1928f*) and posterior (*Butler, 1935*) portions of the blastoderm, although present in the anterior three quarters as well (*Butler, 1935*).

During the first 10 hours of incubation, heart-forming material is still widespread, but it tends to become more concentrated posteriorly (*Dalton, 1935; Spratt, 1942*). With the formation and elongation of the primitive streak, this tendency is accentuated; it is possible that only the posterior half of the blastoderm contains future cardiac tissue at the stage of the long primitive streak (*Spratt, 1942*). Rudnick (1938c) demonstrated that the areas of cardiac potency move toward the posterior midline from either side during early primitive streak stages. Shortly before the stage of the definitive primitive streak, the capacity for heart formation is greatest within the streak itself, apparently because the future heart-forming cells are being invaginated from the surface of the blastoderm into the streak.

At the stage of the definitive primitive streak and thereafter, the invaginated heart material is found in the mesendodermal portion of the blastoderm (*Rudnick, 1935, 1938a*) on either side of the streak. Heart-forming capacity, which is especially strong at the level of the primitive node (*Rudnick, 1938a*), is now localized in a bilateral area that extends from 0.2 mm. anterior to the primitive pit (*Hunt, 1932*) to about 0.4 mm. posterior to it (*Rawles, 1943*).

With the appearance of the head-process and the regression of the node, the heart areas elongate (*Rawles, 1936*). Their anterior limits are fixed at the level of the cephalic tip of the head-process, and their posterior limits move backward with the node, maintaining a position 0.3 to 0.4 mm. caudal to the primitive pit. Each area is approximately 0.6 mm. wide, beginning 0.2 mm. from the midline and extending to a point approximately 0.8 mm. from it, or very close to the border of the area opaca (*Rawles, 1943*). On either side, heart-forming capacity is still strongest at the node level and diminishes anteriorly, posteriorly, and laterally (*Willier and Rawles, 1935; Rawles, 1943*).

The bilateral disposition of the heart-forming material after the stage of the definitive primitive streak is indicated by the appearance of two hearts when the union of the right and left cardiac area is prevented experimentally (*Warynski and Fol, 1884; Warynski, 1886*). The phe-



nomenon of double heart formation has been observed in the posterior half of definitive primitive streak blastoderms divided transversely through the primitive pit and then grown *in vitro* (Waddington, 1932), and also in head-process blastoderms injured in the midline immediately anterior to the tip of the head-process (Szepsenwol, 1933a, 1934; Wolff, 1933; Waddington and Cohen, 1936).

### The Establishment of the Tubular Heart

When the cardiogenic material becomes visibly differentiated, it still occupies two separate areas, one on either side of the midline. The bilaterality of the first recognizable heart tissue was noted by many embryologists of the nineteenth century (Dareste, 1866, 1876, 1877; His, 1868, pp. 84–85; Afanasieff, 1871; Balfour, 1873c).

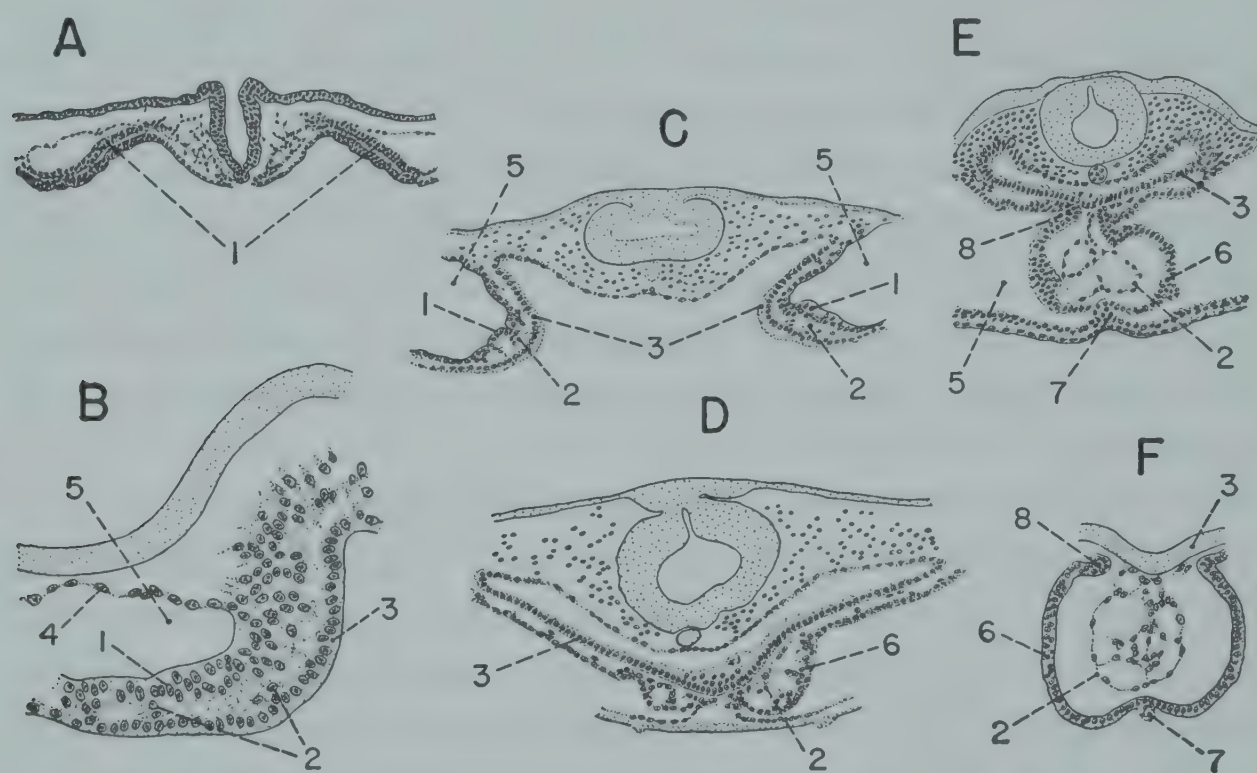
The earliest indication of heart formation is seen in the chick at approximately the 2-somite stage (Gräper, 1907) as a thickening of the splanchnic mesoderm in the medial part of the floor of each amniocardiac vesicle, or cephalic portion of the coelom. The splanchnopleure of this region almost invariably differentiates as cardiac tissue when removed and grown *in vitro* (Olivo, 1928b, 1928e, 1928f).

The bilateral thickenings of the splanchnic mesoderm will give rise to the myocardial tissue, which makes up the muscular wall of the heart. In the chick the first endocardial (endothelial) cells appear between the endoderm and the thickened mesoderm at some time between the 3-somite and the 5-somite stages (Gasser, 1877b; Rückert and Mollier, 1906; Sabin, 1920); in the duck (*Anas platyrhynchos*), they can be seen at the 5-somite stage (Boerner-Patzelt, 1931). The primitive endothelial cells of the heart arise in exactly the same manner as those of the blood vessels (see Chapter 8); that is, by the vacuolization of solid angioblastic aggregates of mesodermal origin (Klein, 1871; Sabin, 1920). The primitive endocardial cells are continuous anteriorly with the angioblastic strands representing the ventral aorta, and posteriorly with the anlagen of the omphalomesenteric veins.

The early stages in the development of the heart are intimately correlated with the closure of the fore-gut, for the endoderm beneath the heart primordia becomes the floor of the pharynx. Originally, the heart primordia lie almost horizontally (Fig. 268-A). The formation of the intestinal trough (see Chapter 6) provides the amniocardiac vesicles with vertical medial walls, of which the heart primordia are a part (Fig. 268-B). The heart primordia now start to bulge laterally as cavitation begins in their endothelial components. (This process can still be seen later at caudal levels of the heart, as shown in Fig. 268-C.) As fore-gut closure progresses caudad, the medial walls of the amniocardiac vesicles bend sharply toward the midline and begin to approach each other, carrying the heart primordia with them (cf. Fig. 268-C). At the 4- to 5-somite stage, the two folds come



into contact in the midline, and their endodermal components fuse. As a result the two sheets of endoderm originally lying beneath the heart primordia form the floor of the fore-gut; and the two halves of the heart, now luminate (*Gasser, 1877b*), lie next to each other beneath the fore-gut, thus in the reverse of their original relationship to the gut endoderm. The endoderm of the pharyngeal floor separates completely from the endoderm of the splanchnopleure, but the myocardial portion of the heart remains in continuity with the splanchnic mesoderm, for the present. Together, the two folds of mesoderm connecting the heart with the splanchnopleure form a membrane known as the ventral mesocardium.



**Fig. 268.** Successive stages in the formation of the double-walled tubular heart from bilateral cardiac primordia in the avian embryo, as seen in cross sections. (Redrawn with modifications A, after Gräper, 1907; B to F, after Rückert and Mollier, 1906.)

A, section of 3-somite gull (*Larus* sp.) embryo ( $\times 25$ ); B, part of section of 3-somite chick embryo ( $\times 150$ ); C, section of 6-somite chick embryo, caudal to the anterior intestinal portal ( $\times 80$ ); D, section of 6-somite chick embryo, cranial to the anterior intestinal portal ( $\times 80$ ); E, section of 8-somite chick embryo ( $\times 80$ ); F, section of 9-somite chick embryo in region where the ventral mesocardium has ruptured ( $\times 100$ ).

1, thickened splanchnic mesoderm (heart primordia); 2, endothelial (endocardial) cells; 3, gut endoderm; 4, somatic mesoderm; 5, pleuroperitoneal cavity; 6, myoepicardium; 7, ventral mesocardium; 8, dorsal mesocardium.

The approximation of the heart primordia continues in the same manner, progressing caudad. At the 5- to 6-somite stage (Fig. 268-D), the still paired heart anlagen are found under a longer portion of the closed fore-gut. Soon the two halves of the heart assume a more ventromedial position, and, as they do so, the endothelial tubes come into contact with each other in the midline (Fig. 268-E).

The first step in the formation of a single tube from the double heart anlagen occurs at the 7- to 8-somite stage (*Gasser, 1877b*), when the



ventral mesocardium becomes a single median strand of cells (cf. Fig. 268-E). This begins to rupture at the midcardiac level during the 9-somite stage (Gräper, 1907; Patten, 1922), and has almost completely disappeared by the 10-somite stage (cf. Fig. 272-A<sub>1</sub>). As the ventral mesocardium ruptures, the ventral myocardial walls of the heart become continuous across the midline (Fig. 268-F), as do the right and left amniocardiac vesicles, which now form the pericardial cavity. Fusion of the two endocardial tubes into one begins almost immediately, at the stage of 9 somites (Gräper, 1907) to 10 or 11 somites (Gasser, 1877b), or, in the duck (*Anas platyrhynchos*) embryo, perhaps not until the 12- to 13-somite stage (Boerner-Patzelt, 1931).

Dorsally, the heart tube begins to be constricted off at the 10- to 14-somite stage (Bruno, 1918; Chang, 1931a), or somewhat later than ventrally. At first, the endocardial cells have a wide contact with the ventral wall of the fore-gut (cf. Fig. 268-D), over which they proliferate angioblasts that probably participate in the formation of blood vessels (Chang, 1931a). Then, as the heart primordia move ventromedially, their myocardial components become suspended beneath the fore-gut by two folds of mesoderm which gradually approach the midline from either side (cf. Fig. 268-E) to form the dorsal mesocardium. The dorsal mesocardium differs from the ventral mesocardium, however, in that its folds include between them a continuation of the inner, endothelial heart tube, firmly attached to the ventral surface of the fore-gut (Fig. 269-B<sub>1</sub>). At the stage of about 12 somites (cf. Fig. 272-B<sub>2</sub>), both the myocardial and endocardial components of the dorsal mesocardium become detached from the fore-gut at the midcardiac level (Patten, 1922). Rupture of the membrane progresses so rapidly that only its cranial and caudal portions remain at the 16- or 18-somite stage (Fig. 269; cf. Fig. 272-C<sub>2</sub>). The cranial portion disappears shortly thereafter, leaving the caudal portion to persist as the permanent mesocardium (cf. Fig. 272-D<sub>2</sub>). It is possible that the endothelial cells of the mesocardium proliferate dorsally to give rise to the pulmonary vein (see Chapter 8).

The endocardial and myocardial portions of the tubular heart are separated by a space filled with a clear, homogeneous, gelatinous substance (Masius, 1889; Szily, 1904, 1908; Kurkiewicz, 1910; Johnstone, 1924; Baitzell, 1925; Garrault, 1934; Chang, 1932; Barry, 1948; Patten, Kramer, and Barry, 1948) which was termed "cardiac jelly" by Davis (1924). The fibers sometimes seen within it are probably produced by fixation (Kurkiewicz, 1910; Bruno, 1918; Baitzell, 1925). The cardiac jelly adheres so closely to the inner and outer cardiac tubes that the latter are separable with difficulty (Davis, 1924; Baitzell, 1925); and it does not escape even when the heart is sectioned (Szily, 1908; Garrault, 1934). It is quite probable that the resiliency and incompressibility of this substance give it physiological



importance during the period between the initiation of the heartbeat and the development of valves. Without the intervention of a space filled with material such as cardiac jelly, the tubular heart could not pump any significant amount of blood because it would be incapable of changing its diameter sufficiently. Cardiac jelly apparently serves to transmit the force of contraction from the myocardial "sleeve" of the early heart radially down against the inner endothelial tube (Boerner-Patzelt, 1931; Barry, 1948). Figure 270 gives an indication of the manner in which cardiac jelly probably facilitates the pumping of blood.

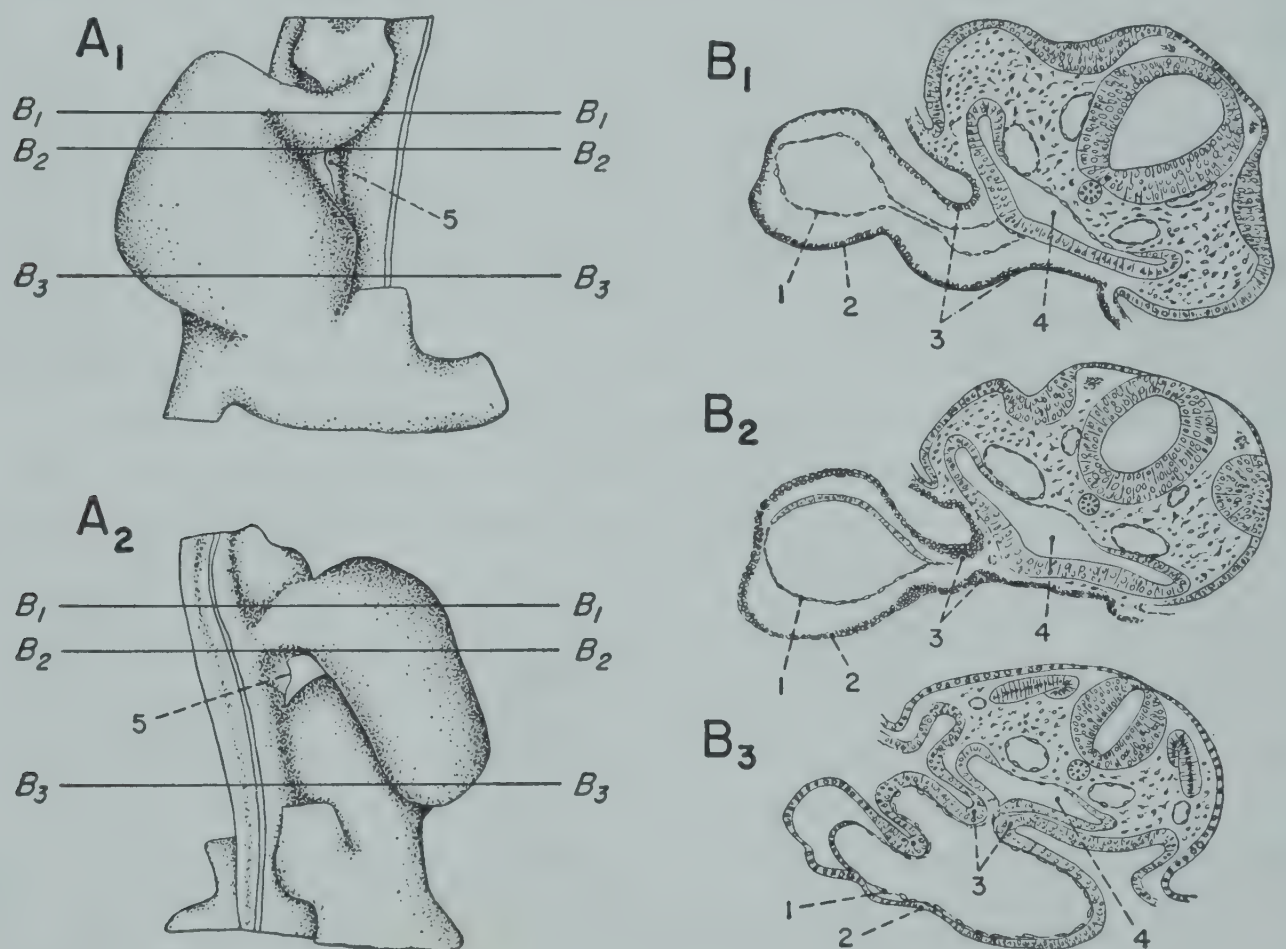


Fig. 269. Location and structure of the dorsal mesocardium in the 18-somite chick embryo. (Redrawn with modifications after Chang, 1931a.)

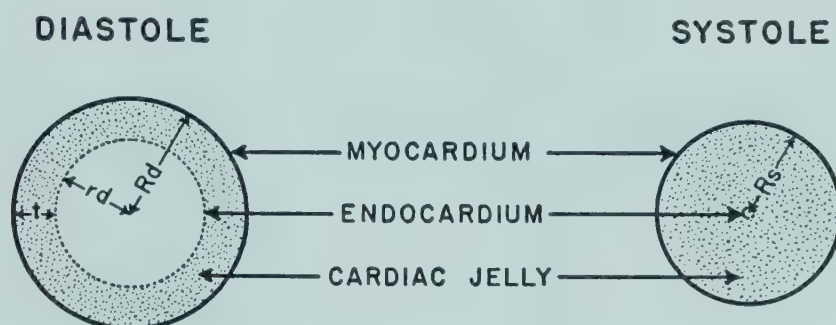
A<sub>1</sub>, ventral view and A<sub>2</sub>, lateral view of the entire heart ( $\times 35$ ); B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, cross sections of the heart at the level of the bulbus, the ventricle, and the sinus venosus, respectively, as indicated by lines in A<sub>1</sub> and A<sub>2</sub> ( $\times 50$ ).

1, endocardium; 2, myocardium; 3, dorsal mesocardium; 4, lumen of fore-gut; 5, remnant of dorsal mesocardium.

The heart tube at the 9- to 12-somite stage represents only the ventricle and the bulbus (the region between the ventricle and the truncus arteriosus). The material that is to provide the remainder of the heart lies along the roots of the paired omphalomesenteric veins (Patten and Kramer, 1933). The anteriormost sections of these veins now become confluent and are added to the heart tube, which is thus lengthened caudally (Baer, 1828b; Balfour, 1873c; Rückert and Mollier, 1906; Gräper, 1907). According to Patten and Kramer (1933), the material for the atrium (fore-



runner of the auricles) has been incorporated into the heart by the 16-somite stage. As reference to Fig. 272 and Fig. 275 will show, the cephalic bulge present in the root of the left omphalomesenteric vein at the 10-somite stage seems to have moved into the heart by the 16-somite stage, to take the position of the atrium caudal to the ventricular region (*Johnstone, 1925*). At the 19-somite stage, fusion of the omphalomesenteric veins begins to involve a still more caudal level, representing the sinus venosus (*Patten and Kramer, 1933*), which is closely associated with the right auricle in the adult heart. It is possible, however, that all this cardiac material is added somewhat earlier, at least on the left side. *Kum  (1935)*



*Fig. 270.* Diagrammatic cross sections of the tubular heart at diastole and at systole, to show the part probably played by the cardiac jelly in narrowing the lumen of the heart during contraction. The drawings indicate a 20 per cent shortening of the circumference of the heart at systole. (Redrawn after *Barry, 1948.*)

$R_d$ ,  $R_s$ , radius of outer myocardial tube at diastole and systole, respectively;  $r_d$ , radius of lumen at diastole;  $t$ , thickness of cardiac jelly at diastole.

found that the ability of the root of the left omphalomesenteric vein to form heart muscle in chorioallantoic grafts is lost after the 14-somite stage. The cardiogenic power of the right vein root, however, increases between the 8- and 16-somite stages. Heart-producing capacity, therefore, may be asymmetrically localized in the two vein roots, so that the center of this capacity in the left root shifts anteriorly at an earlier time than the center in the right root. This asymmetry is perhaps attributable to the larger size of the left omphalomesenteric vein.

As the endothelium of the omphalomesenteric veins is added to the heart, there is a downgrowth of myocardium over it (*Johnstone, 1925*; *Patten and Kramer, 1933*). The outer heart tube is applied fairly closely to the endothelium, without the intervention of cardiac jelly (*Masius, 1889*).

### Bending and Torsion of the Heart

At the 9- or 10-somite stage, the straight heart tube begins to elongate more rapidly than the pericardial cavity containing it, and, to accommodate itself to the available space, it is forced to bend. This bending process stretches the dorsal mesocardium and causes it to rupture. The midportion



of the heart is thus set free in the cavity, but the anterior and posterior ends remain in fixed positions, held by the ventral aorta and the caudal remnant of the dorsal mesocardium, respectively. As Bremer (1928*b*) remarked, the heart now behaves like a length of rubber tube held rigidly at both ends. Bringing the ends closer together—equivalent to lengthening the heart tube within a confining space—causes the tube to bend (Fig. 271-*A*<sub>1</sub> and *A*<sub>2</sub>).

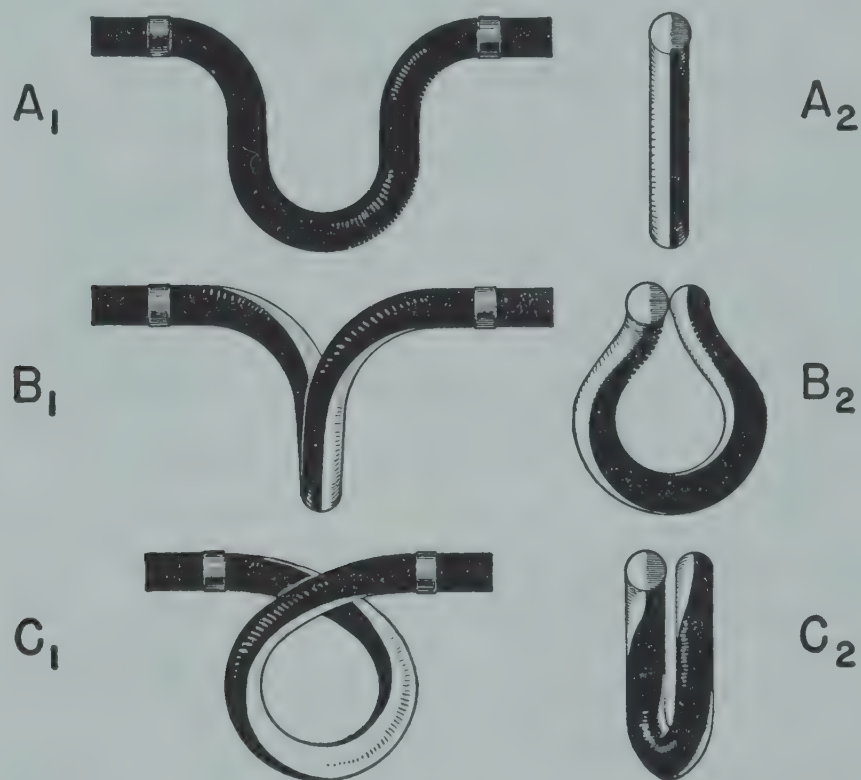


Fig. 271. The development of a dextral spiral in the chick embryo heart illustrated by the bending of a rubber tube as its ends are brought closer together. This shows how similar changes in shape take place in the growth of the tubular heart. (Redrawn with modifications after Bremer, 1928*b*.)

*A*<sub>1</sub>, *A*<sub>2</sub>, lateral and sagittal views show that the tube bends in the sagittal plane; *B*<sub>1</sub>, *B*<sub>2</sub>, the bend swings into the transverse plane; *C*<sub>1</sub>, *C*<sub>2</sub>, the bend is transformed into a loop in the sagittal plane.

The heart cannot bend ventrally because of the resistance offered by the yolk beneath; instead, it curves to the right (Fig. 272). It is not clear why the curvature is directed this way rather than to the left, but the right heart bend occurs in all vertebrates above and including *Elasmobranchi* and therefore appears to be deeply rooted in phylogeny (Patten, 1922). The primary nature of the dextral curvature is indicated also by the fact that the right wall of the heart is more dilated than the left wall before bending is actually initiated. Thus the tubular heart appears curved to the right in the 9-somite chick (cf. Fig. 275-A) and the 12-somite duck, *Anas platyrhynchos* (Boerner-Patzelt, 1931) and turkey, *Meleagris gallopavo* (Phillips and Williams, 1944). This unequal dilation cannot be due to the impingement of any blood stream, since circulation has not yet begun



(Patten, 1922). It has been found that the normal curvature of the heart does not develop when the egg's content of nicotinic acid is artificially increased (Hansborough, 1947b).

As the heart continues to grow, it forms an increasingly deep U-shaped curve to the right (Fig. 272). The curve reaches its maximum depth at approximately the 18-somite stage (Patten, 1922).

By this time, the development of the heart has begun to be affected by the torsion of the embryo's body. About at the 16-somite stage, the left

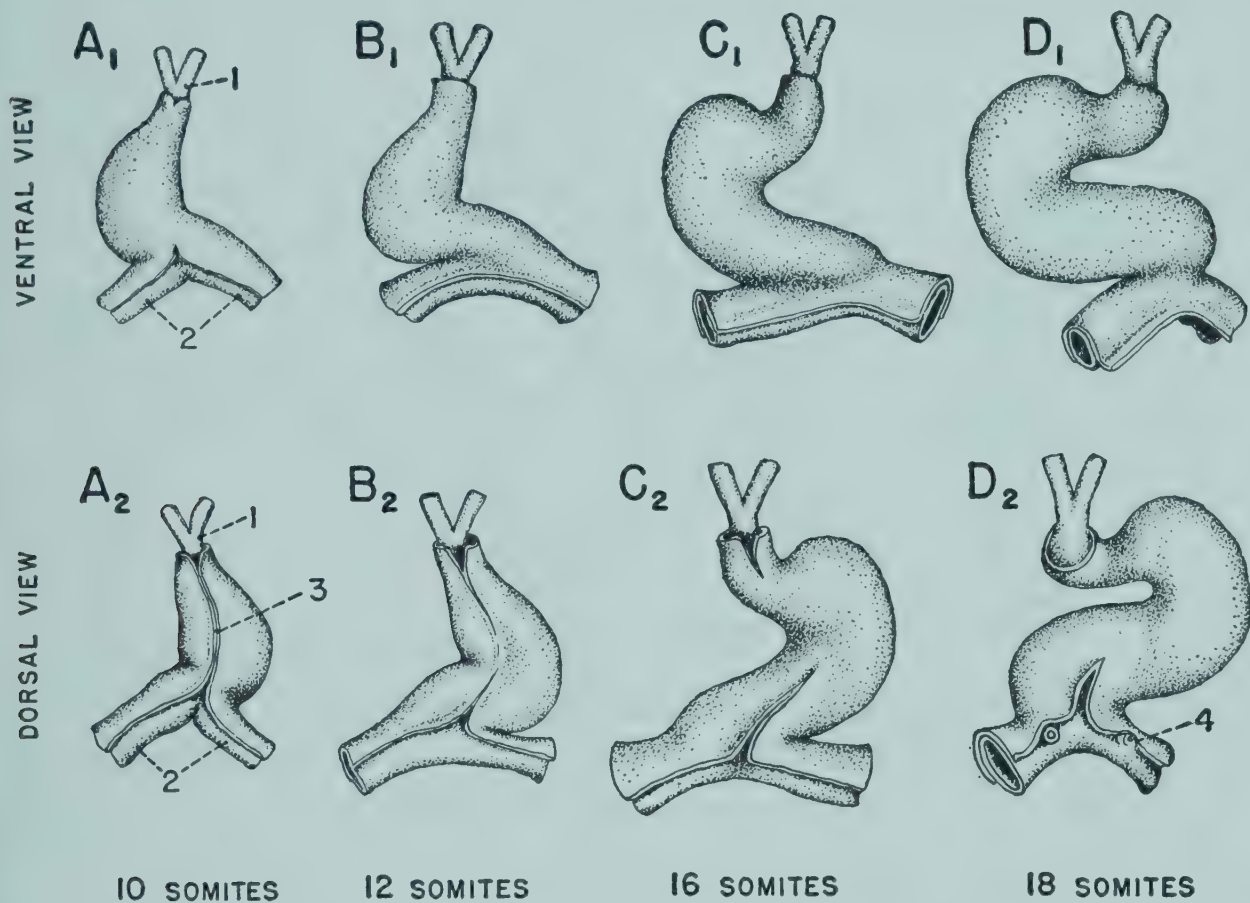


Fig. 272. The development of a dextral curvature in the heart of the chick embryo between the 10- and 18-somite stages, as seen in dorsal and ventral views. (Selected and redrawn from Patten, 1922.) All  $\times 25$ .

1, ventral aortic root; 2, omphalomesenteric veins; 3, dorsal mesocardium; 4, duct of Cuvier.

side of the head starts to turn toward the yolk (cf. Fig. 275-D). When the leftward rotation of the body reaches the cardiac level, the heart also begins to turn counterclockwise. The cephalic end of the heart is thus carried away from the yolk (Patten, 1922); that is, it comes to lie closer to the observer, or farther to the embryo's right, than the caudal end of the heart.

On the other hand, it is possible that the rotation of the heart is a primary developmental factor and that it is largely responsible for, rather than dependent upon, the torsion of the body. Weber (1902e) remarked that the right omphalomesenteric vein at the 10- to 12-somite stage, already enters the heart of the duck (*Anas platyrhynchos*) embryo at a more dorsal level than the left vein. Waddington (1937a) observed that



torsion of the head did not occur in chick embryos, grown *in vitro*, whose hearts he had removed at stages of 7 to 12 somites. The cranial flexure developed normally, however.

The cranial flexure (the bending of the head in the midbrain region, so that the cephalic end of the forebrain is eventually directed caudad) has a definite effect on the configuration of the heart tube. Between the 22- and the 29-somite stages (cf. Fig. 275-G, H, and I), the flexure of the head prevents the distance between the attached ends of the heart from increasing and, in fact, shortens it slightly; yet the heart is still growing at a rapid

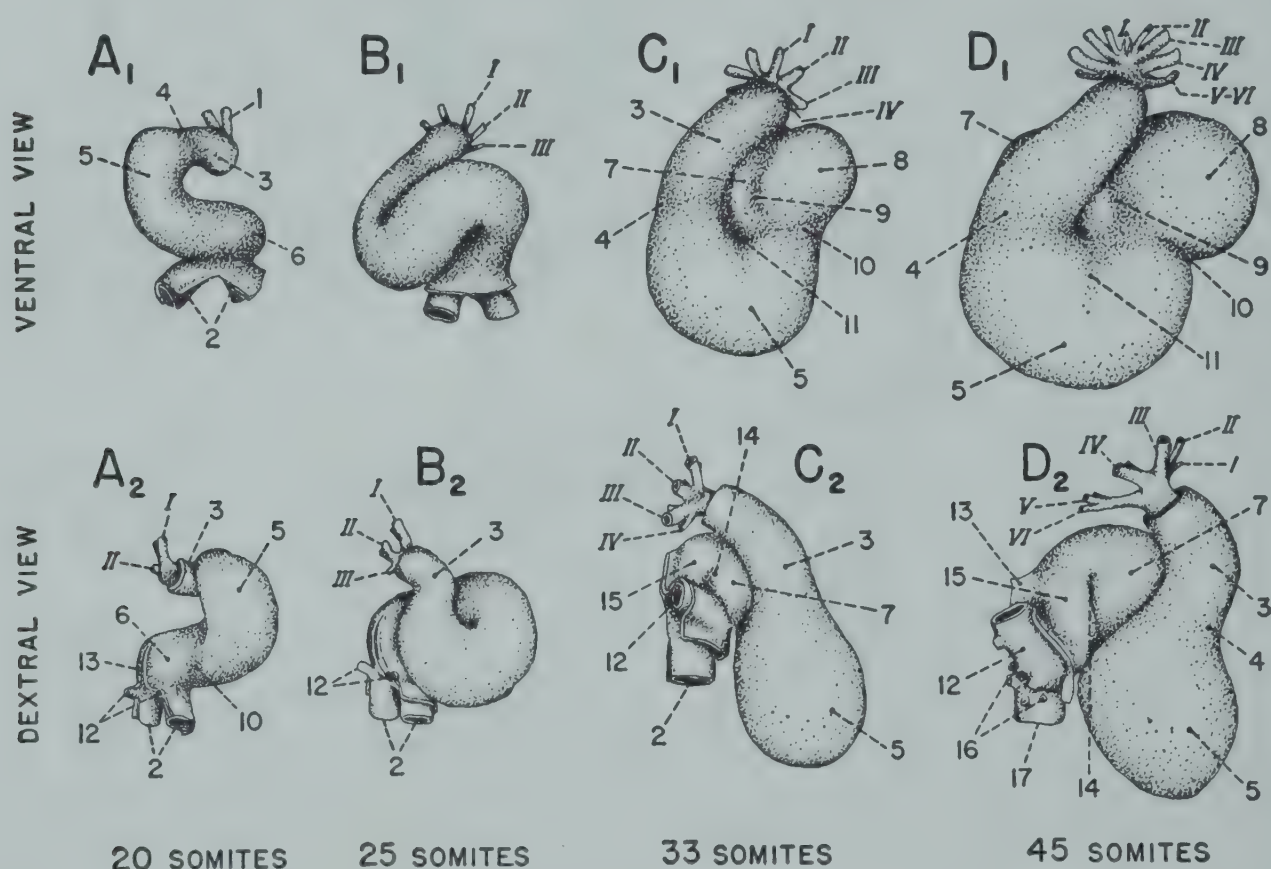


Fig. 273. External changes in the shape of the chick embryo's heart between the 20- and 45-somite stages, shown in ventral and dextral view. (Redrawn after Patten, 1922.) All  $\times 15$ .

1, ventral aortic root; 2, omphalomesenteric veins; 3, bulbus cordis; 4, bulboventricular constriction; 5, ventricle; 6, sinoatrial region; 7, right atrium; 8, left atrium; 9, interatrial groove; 10, atrioventricular constriction; 11, interventricular groove; 12, duct of Cuvier; 13, dorsal mesocardium; 14, sinoatrial constriction; 15, sinus venosus; 16, hepatic sinusoids; 17, fused omphalomesenteric veins; I, II, III, IV, V, VI, aortic arches.

rate. As a result, the U-shaped, slightly spiraled heart tube is rapidly transformed into a loop, with its cephalic and caudal ends close together, the cephalic end still lying to the right of the caudal end (Fig. 273-A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub>, and B<sub>2</sub>; Fig. 274-B). The rubber tube of Fig. 271-B<sub>2</sub> and Fig. 272-B<sub>1</sub> also forms a loop when its ends are still more closely approximated, as shown in Fig. 271-C<sub>1</sub> and C<sub>2</sub>. The heart at this stage has frequently been described as S-shaped, although it is recognizable as such only in a true ventral view (Fig. 273-B<sub>1</sub>). The S-shaped heart is seen in duck (*Anas platyrhyn-*



*chos*) and turkey (*Meleagris gallopavo*) embryos of 20 to 22 somites (Boerner-Patzelt, 1931; Phillips and Williams, 1944).

The process of torsion reaches the level of the caudal end of the heart about at the 29-somite stage (cf. Fig. 275-I). Increasingly caudal portions of the heart, therefore, are being rotated during the period of cardiac loop formation. As Bremer (1932) pointed out, the rotation of the caudal end of the heart may be due merely to the coiling of the heart tube, which causes the cephalic end to press against the right side of the caudal end. The cardiac loop and the rotation of its caudal end occur in animals that do not undergo torsion of the body.

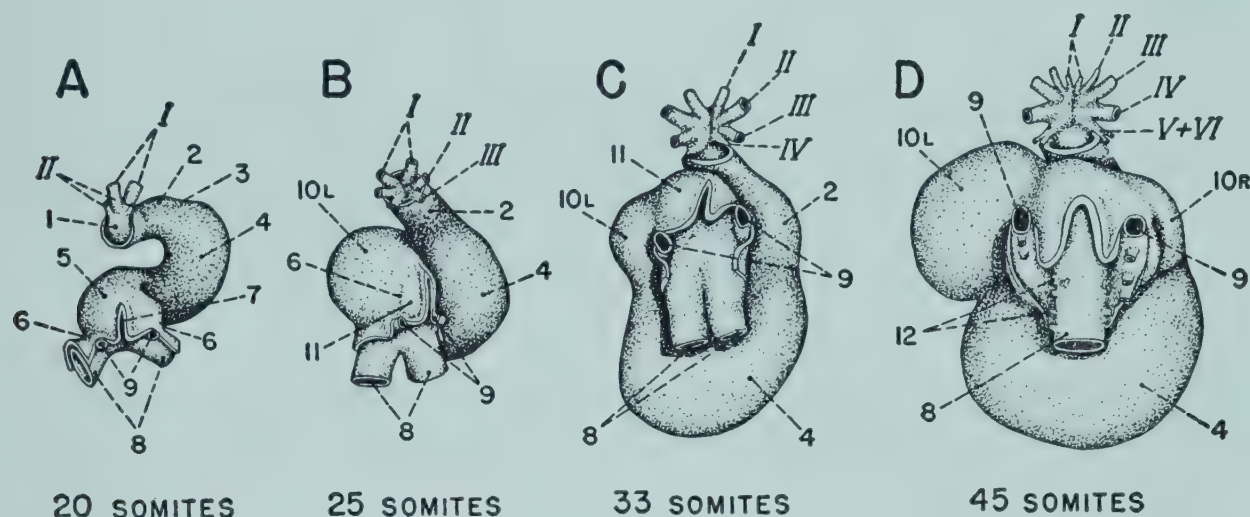


Fig. 274. Dorsal views showing changes in the shape of the chick embryo's heart between the 20- and 45-somite stages. (Redrawn after Patten, 1922.) All  $\times 15$ .

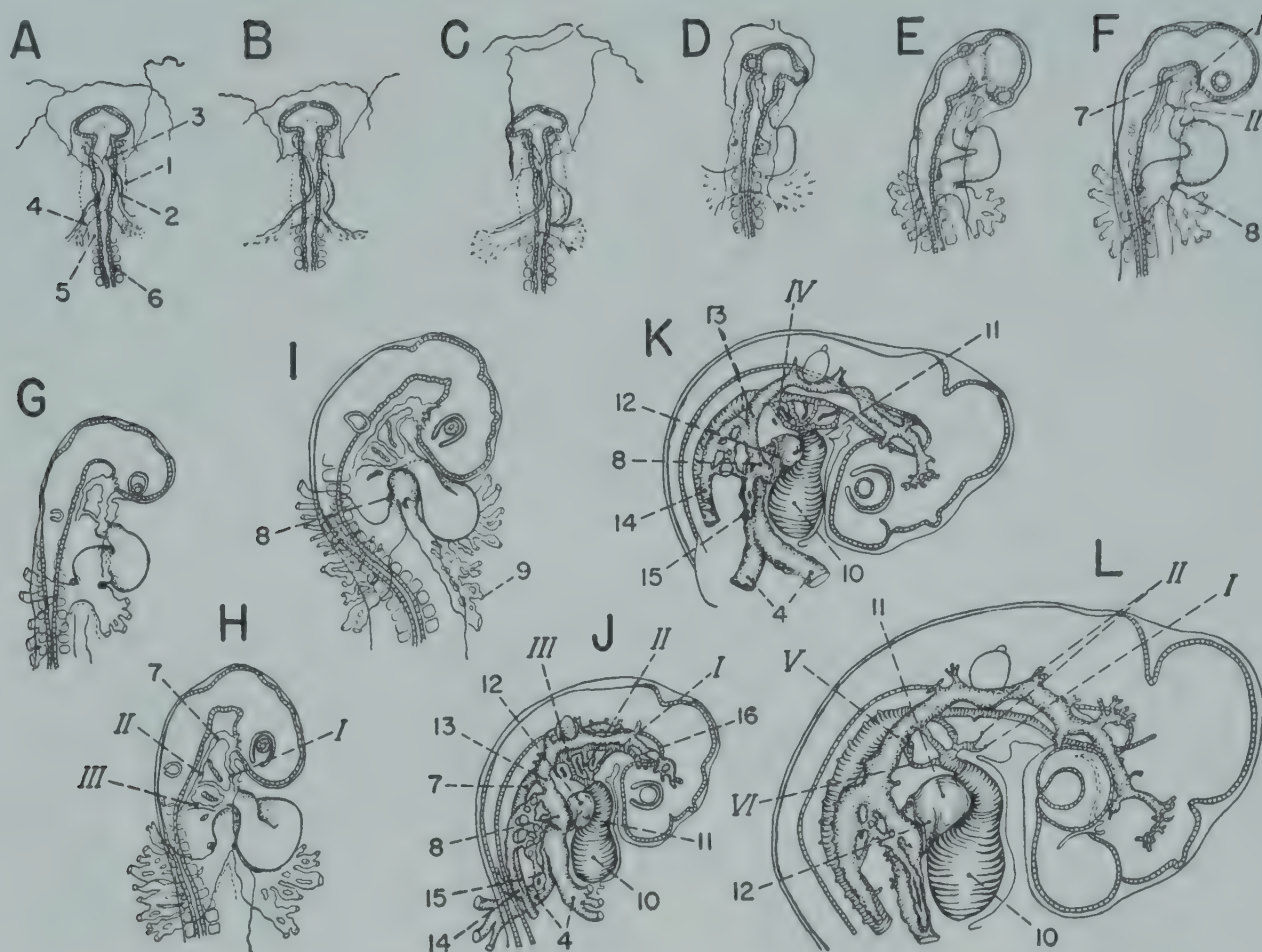
1, ventral aorta; 2, bulbus; 3, bulboventricular constriction; 4, ventricle; 5, sinoatrial region; 6, sinoatrial constriction; 7, dorsal mesocardium; 8, omphalomesenteric veins; 9, duct of Cuvier; 10<sub>R</sub>, 10<sub>L</sub>, right and left atrium; 11, sinus venosus; 12, stumps of hepatic sinusoids; I, II, III, IV, V, VI, aortic arches.

The combined effect of loop formation and rotation is to swing the heart ventrad and toward the sagittal plane of the body. During the chick's third incubation day, the position of the heart is further modified under the influence of the rapidly developing cranial flexure. The anterior end of the head pushes the heart caudad and dorsad through an arc of about  $30^\circ$  (Fig. 275-I, J, K, and L), so that the ventricular region of the heart becomes its most caudal portion (Patten, 1922).

After the chick's fourth day of incubation, the bulbus and right ventricle rotate around the longitudinal axis of the heart so that the bulbus moves into a median ventral position from its former location ventral to the right atrium (Masius, 1889; Langer, 1894; Hochstetter, 1906).

At 6 to 8 days a membranous sac, the pericardium, is formed around the heart, enclosing the pericardial cavity. The pericardium is derived from two main sources: the body wall, and the portion of the septum transversum which forms the anterior membrane of the liver.





**Fig. 275.** Changes in the relationship of the developing heart to other structures in the chick embryo's body from the 9- to the 45-somite stage, inclusive. (Redrawn after Patten, 1922.)

A, 9 somites (29 hours); B, 10 somites (30 hours); C, 12 somites (32 hours); D, 16 somites (38 hours); E, 18 somites (40 hours); F, 20 somites (42 hours); G, 22 somites (44 hours); H, 25 somites (47 hours); I, 29 somites (53 hours); J, 33 somites (65 hours); K, 38 somites (76 hours); L, 45 somites (100 hours). All  $\times 8$ .

1, myoepicardium; 2, endocardium; 3, ventral aorta; 4, omphalomesenteric vein; 5, anterior intestinal portal; 6, neural tube; 7, dorsal aorta; 8, duct of Cuvier; 9, vitelline veins; 10, ventricle; 11, right atrium; 12, sinus venosus; 13, anterior cardinal vein; 14, posterior cardinal vein; 15, allantoic vein; 16, internal carotid artery; I, II, III, IV, V, VI, aortic arches.

### The Regional Differentiation of the Heart

Very early in its development, the heart is subdivided into its primitive regions, which, from the caudal to the cephalic end, are the sinus venosus, the atrium, the ventricle, and the bulbus cordis (continuous cephalically with the truncus arteriosus). The differentiation of these regions appears to be an autonomous process. It has been found to occur in double hearts that develop when the heart primordia are prevented from uniting by the infliction of a median injury anterior to the head-process (Wolff, 1933).

As previously noted, the first portion of the heart tube to be established represents the combined bulbus and ventricle, the ventricle being recognizable by its greater diameter even in the beginning. The "segmentation" of the heart becomes evident as superficial grooves or constrictions make their appearance between localized expansions. A certain regional subdivision is detectable before the cardiac loop starts to form, and all the primi-



tive regions of the heart are distinguishable by the 25-somite stage. The incipient division of the atrium and ventricle into right and left halves is apparent externally at (or soon after) the end of the chick's third incubation day, and the heart approximates the adult form before the end of the eighth day.

At the 16- to 18-somite stage, the primitive ventricle begins to be demarcated from the bulbus and atrium by the vaguely discernible bulbo-ventricular and atrioventricular grooves, respectively. These grooves become clearly apparent at the 20-somite stage, when the ventricle starts to expand (Fig. 274-A; cf. Fig. 273-A<sub>1</sub> and A<sub>2</sub>). By the 29-somite stage, the ventricle has lost its former U-shaped configuration and has become quite saccular. The first external indication of its internal division into right and left chambers appears at the 33-somite stage (cf. Fig. 273-C<sub>1</sub>), when a very slight groove, originating on its inner curvature, extends caudad and to the right on its ventral surface (*Hochstetter, 1906; Patten, 1922*). As this groove becomes deeper, it divides the ventricle unequally, so that the left side is larger than the right. The diagonal course of the interventricular sulcus makes it obvious that the originally caudalmost part of the cardiac loop becomes the left ventricle. During the fifth incubation day, the right side of the ventricle starts to expand, and both the interventricular groove and the bulbus undergo an apparent displacement to the left, the result, actually, of rotation. Simultaneously, a groove appears on the dorsal surface of the ventricle (*Hochstetter, 1906*). During the later development of the heart, the interventricular sulcus becomes less apparent as the muscular wall of the heart grows thicker.

The bulboventricular groove gradually disappears as the proximal part of the bulbus is incorporated into the ventricle. The distal part of the bulbus then becomes the proximal part of the truncus arteriosus. The truncus arteriosus is eventually divided into two tubes, the pulmonic and aortic trunks, by two parallel spiral furrows that first appear distally and grow heartward, deepening as they develop (*Tonge, 1869*). The pulmonic trunk leads off from the right ventricle, the aortic trunk from the left. The separation of the truncus into two vessels reflects internal structural changes which are not complete until the chick's seventh or eighth incubation day (*Bremer, 1928a*).

The atrial region, incorporated into the heart at the 16-somite stage, begins to expand laterally at the 20-somite stage (Fig. 274-A; cf. Fig. 273-A<sub>2</sub>). Its expansion, which is more marked on the left side, deepens the atrioventricular groove. When the cardiac loop is formed at the 25-somite stage, the bulbus comes to lie across the right side of the atrium (Fig. 274-B; cf. Fig. 273-B<sub>2</sub>). The retarded dilation of this side is thus further inhibited until the 29-somite stage, when the bulbus slips past the atrium. Rapid expansion of the right side of the atrium now ensues (cf. Fig.



273-C<sub>2</sub>), and a longitudinal sulcus appears on the ventrocephalic surface of the atrium, marking the location of the interatrial septum (cf. Fig. 273-C<sub>1</sub>). The bulbus and truncus arteriosus gradually sink down between the two enlarging atria (*Patten, 1922*). The interatrial groove extends dorsad after the fifth day but is eventually obliterated during the final stages of incubation (*Quiring, 1933*).

The sinus venosus, which is probably added to the heart tube after the 16-somite stage, begins to be recognizable as a differentiated region at the 25-somite stage (*Patten and Kramer, 1933*). At this time, two slight grooves can be seen extending over the dorsal surface of the atrium from the points of entrance of the ducts of Cuvier, which mark the caudal boundary

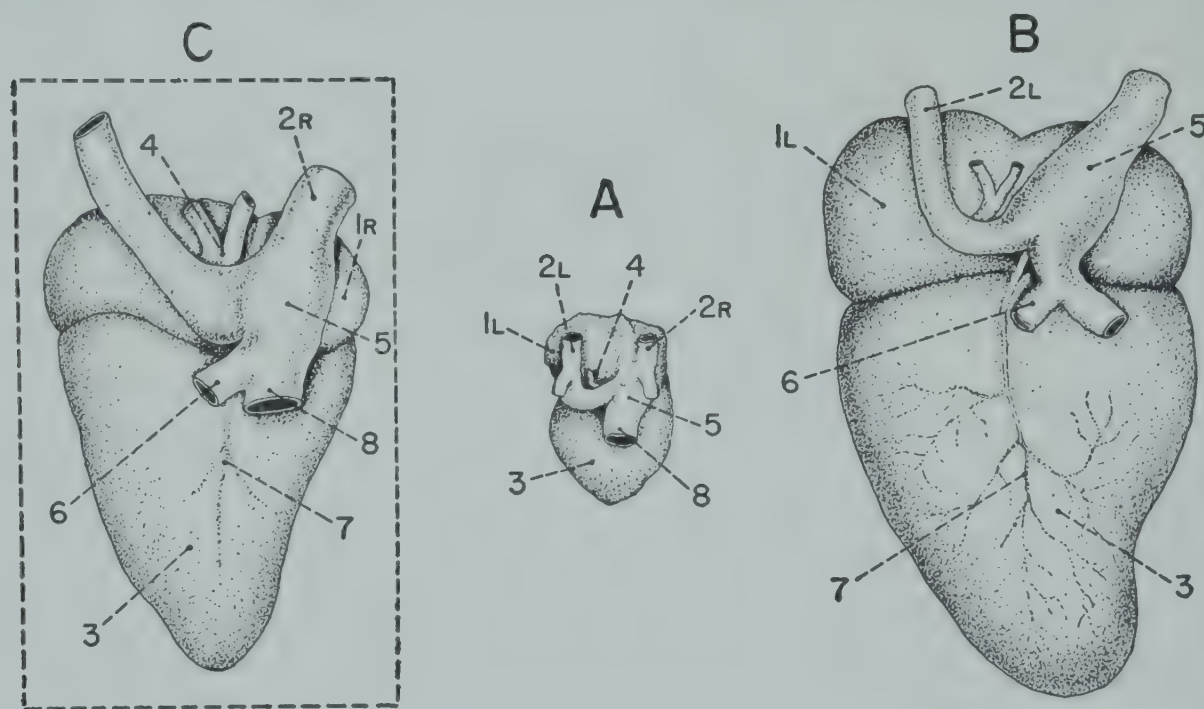


Fig. 276. Changes in the relationship of the left and right precaval veins and the sinus venosus to the atria in the heart of the chick embryo, as seen in dorsal view; the adult chicken heart is shown for comparison. (Redrawn with modifications after *Quiring, 1933*.)

A, at 5 days ( $\times 6$ ); B, at 18 days ( $\times 6$ ); C, adult heart (natural size).

1<sub>R</sub>, 1<sub>L</sub>, right and left atrium; 2<sub>R</sub>, 2<sub>L</sub>, right and left anterior vena cava; 3, ventricle; 4, pulmonary vein; 5, sinus venosus; 6, hepatic vein; 7, coronary vein; 8, posterior vena cava.

of the sinus region (Fig. 274-B; cf. Fig. 273-C<sub>2</sub> and D<sub>2</sub>). The grooves lie at either side of, and parallel to, the caudal remnant of the dorsal mesocardium. The portion of the dorsal atrial wall that lies between the grooves now begins to dilate (Fig. 274-C; Fig. 273-C<sub>2</sub> and D<sub>2</sub>), thus constituting the sinus. Simultaneously, the flexed forebrain begins to crowd the heart caudad and dorsad (cf. Fig. 275-I, J, and K), with the result that the sinus is brought into a dorsal position during the chick embryo's third incubation day (*Patten, 1922*).

Later, the sinus venosus and the proximal part of the right duct of Cuvier appear to be incorporated into the dorsal wall of the right atrium (*Hochstetter, 1906*) as the interatrial furrow becomes continuous dorsally with



the furrow delimiting the sinus from the left atrium (Fig. 274-D). In the chicken, the sinus continues to exist as a distinct entity; and, in the adult heart (Fig. 276-C), a furrow clearly demarcates it laterally from the right atrium (Quiring, 1933).

The left duct of Cuvier remains dorsal to the left atrium, coursing in the atrioventricular furrow before entering the sinus (Hochstetter, 1906). Before the end of the chick's fifth incubation day (Fig. 276-A), the ducts of Cuvier are incorporated into the precaval veins, and the posterior vena cava largely replaces the ductus venosus (or common root of the omphalomesenteric veins). The posterior vena cava opens into the sinus venosus caudally; the right precaval vein enters the sinus cephalodorsally; and the left precaval vein passes over the dorsal wall of the left atrium, bends abruptly to the right, and joins the sinus medioventrally (Quiring, 1933). The pulmonary vein (whose root shifts from the sinus to the left atrium during the chick's third incubation day) makes its exit within the bend of the left precaval vein. Superficially, little change is seen in these relationships at the venous end of the heart from this time until the end of incubation (Fig. 276). There is a considerable increase in the size of the right precaval vein after the seventh incubation day.

#### *The Development of Internal Cardiac Structure*

The differentiation of the interior of the heart is much more complex than external appearances indicate. The proper functioning of the heart depends not only upon muscular contraction but also upon the presence of such internal features as septa and valves, which direct and regulate the flow of blood. The development of these structures transforms the simple heart tube into a four-chambered organ the right and left sides of which do not intercommunicate. Thus the septation of the heart, although not entirely complete until pulmonary respiration begins, consists essentially of a longitudinal division that separates the pulmonic circulation on the right side from the systemic circulation on the left side.

It was suggested by Bremer (1928a, 1932) that the plane of longitudinal division is not sagittal but horizontal. The clue to the situation is given by the fact that the plane of division passes through the truncus arteriosus in such a way that the portion of the truncus leading from the right ventricle remains connected with both sixth aortic arches, while the portion leading from the left ventricle remains connected with the fourth and third arches. It is clear that only horizontal division could achieve this result.

The hydrodynamic action of two spiral blood streams passing through the heart was suspected by Weber (1902e) and is probably responsible for the separation of the pulmonic and systemic circulations. The cardiac septa form between these blood streams, where the centrifugal force of the current is least (Bremer, 1928a, 1932). Although the myocardium is

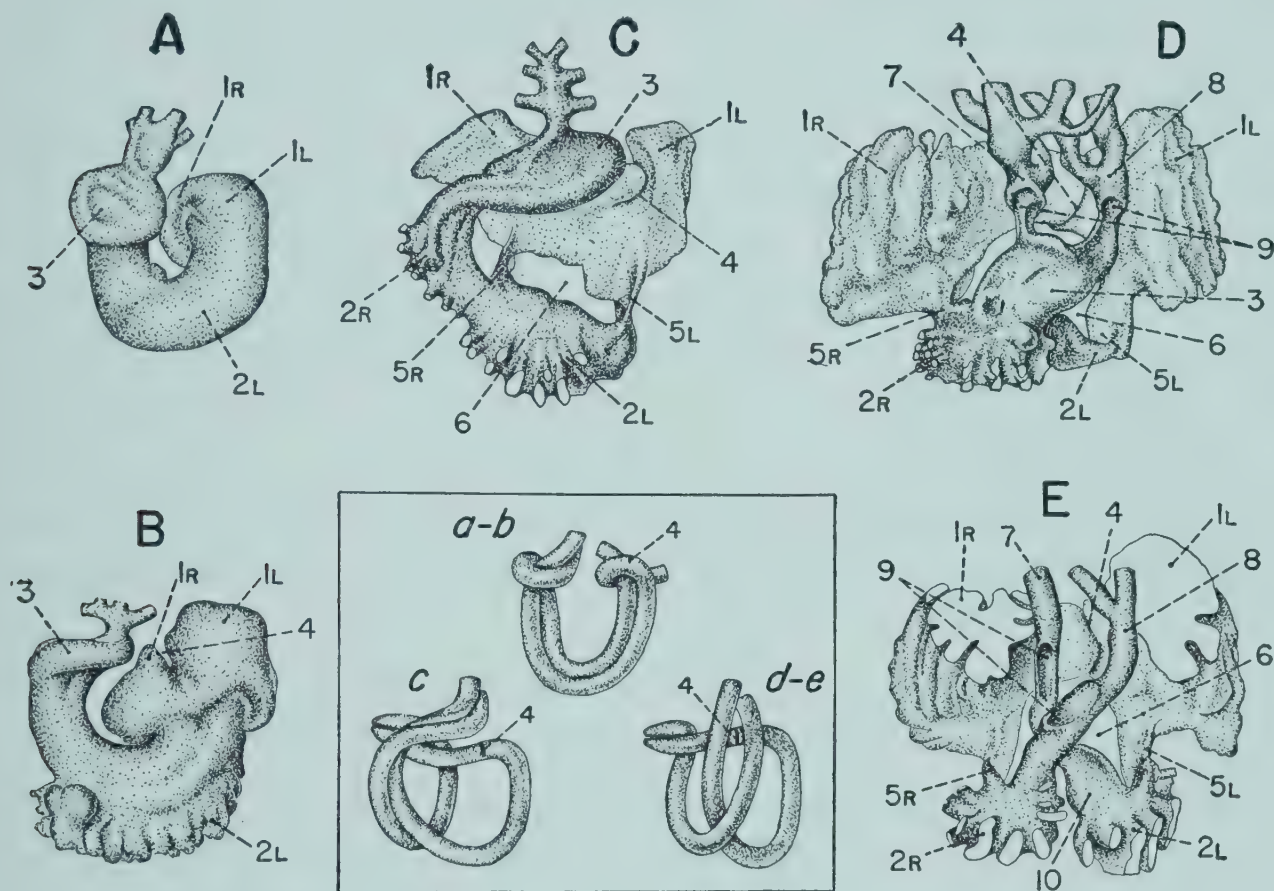


little affected, the cardiac jelly is of such a consistency that it can be molded readily, and it very possibly establishes the pattern along which the septa later develop (*Patten, Kramer, and Barry, 1948*). The septa form by the proliferation of tissue (*Lindes, 1865; Masius, 1889*) into the cardiac jelly. Every septum (except the interatrial) originates as two opposing ridges that grow into the lumen of the heart and meet. Septa appear separately in the bulbotruncus, ventricle, and atrioventricular canal and eventually become continuous, occupying the sagittal plane because of the rotation of the heart. This composite longitudinal septum accurately meets the interatrial septum, which also lies in the sagittal plane and therefore appears to be longitudinal. In actuality, it forms across one of the blood streams in the heart (*Bremer, 1928a, 1932*).

The two blood streams that pass through the heart enter it from the omphalomesenteric veins. At the venous end of the 2-day chick embryo's heart, the atrium and sinus venosus are already rotated slightly to the left. The mouth of the right omphalomesenteric vein, therefore, is closer to the observer than that of the left vein. Blood entering the sinus from the left vein is directed diagonally to the right. It crosses the sinus caudal or ventral to the blood entering from the right vein and then, meeting the rounded right wall of the atrium, is deflected in such a way that it spirals cranially and to the left around the stream of blood from the right vein. It then flows around the greater curvature (or left side) of the ventricular bend and passes out of the heart via the ventral (now cranial) side of the bulbus, in a favorable position for entering the more cranial aortic arches (the third and fourth). The blood stream from the right vein follows the axis of the atrial spiral and the inner curvature of the ventricular bend and then, in the bulbus, turns around the stream from the left vein in a spiral that is the reverse of the atrial spiral. It leaves the heart in such a way that it tends to enter the caudalmost (sixth) aortic arches. In Fig. 277—Insert *a-b*, the two contiguous blood streams are represented by two intertwined tubes, the right ends of which correspond to the venous end of the heart. The black line across the stream from the left vein indicates the future position of the interatrial septum and lies in direct continuity with the future plane of the interventricular septum (which will develop between the two blood streams in the ventricular region). The interatrial septum forms to the right of the mouth of the pulmonary vein, which thereby becomes located in the left atrium and on the systemic side of the heart. This hypothetical representation may be compared with Fig. 277-A and B, which are reproductions of wax casts of the heart cavity from chicks incubated 2 days and 2 days 18 hours, respectively. The resemblance is striking. In Fig. 277-B, the early formation of the atria is obvious, and the multiple pouches at the bottom of the heart curve indicate the beginning of the left ventricular dilation.



In the heart of the 4.5-day embryo, the endocardial cushions have developed in the atrioventricular canal and have widely separated the two streams of blood as they pass into the ventricular region. This separation has entailed the elongation of the transverse arm of the atrial spiral and the movement of the interatrial septum to the right, as well as a loosening of the bulbar spiral. The condition at this stage is shown in Fig. 277—Insert c and C, again representing a hypothetical model and a wax cast of



**Fig. 277.** Changes in the shape of the cavities in the heart of the chick embryo under the hydrodynamic influence of the two spiral streams of blood flowing through the heart. Successive stages in development from the beginning of the third incubation day to the middle of the sixth day are shown by ventral views of wax casts of the heart cavities. Corresponding changes in the course of the two blood streams are represented by the figures in the *Insert*. (Redrawn with modifications after Bremer, 1928a, 1932.)

A, at 50 hours; B, at 66 hours; C, at 108 hours; D, at 120 hours; E, at 135 hours.

1<sub>R</sub>, 1<sub>L</sub>, right and left atrium (the upper portions of the atria have been removed in E); 2<sub>R</sub>, 2<sub>L</sub>, right and left ventricle (the multiple pouches of the ventricles have been cut off in C, D, and E); 3, bulbus; 4, site of the interatrial septum; 5<sub>R</sub>, 5<sub>L</sub>, right and left atrioventricular canals; 6, space occupied in the living heart by the endocardial cushions; 7, aorta; 8, pulmonary artery; 9, semilunar valves; 10, conus arteriosus.

the heart cavity, respectively. In Fig. 277-C, the casts of the atrioventricular canals are narrow bands separated by a wide space filled, in life, by the endocardial cushions. The right ventricle may be seen just proximal to the bulbar spiral. The atria have a broad intercommunication, and the right wall of the left atrium is concave where it borders the leftward bulging interatrial septum.

In later stages, there is a further separation of the two streams of blood



which, after 5 days' incubation, remain contiguous only in the right atrium and at their point of exit from the ventricles to the bulbus. The bulbar spiral has become almost, but not quite, completely uncoiled, so that the blood streams merely cross upon leaving the ventricles and then separate. The model of this stage, shown in Fig. 277—Insert *d-e*, is to be compared with the casts of the heart cavity from embryos incubated 5 days and 5.5 days, reproduced in Fig. 277-*D* and *E*, respectively. It can be seen that the uncoiling of the bulbar spiral corresponds to the division of the bulbotruncus into aortic and pulmonic trunks. This division is complete in the 5.5-day embryo except in a small region not visible in the figure.

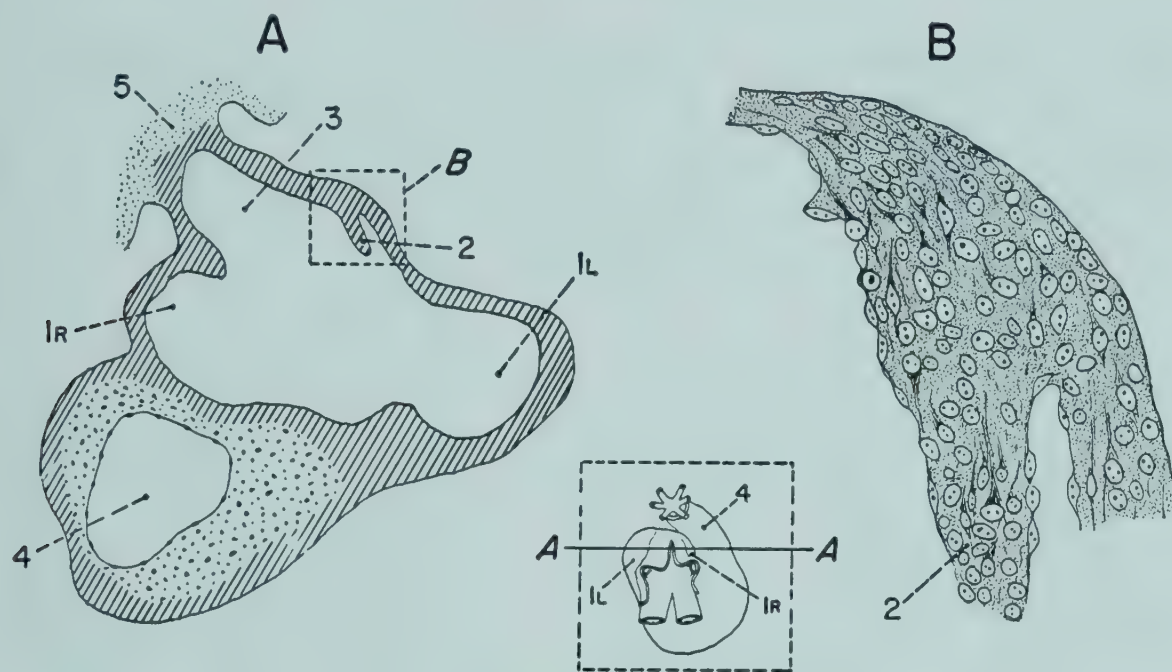
**The Atria (auricles).** The originally single atrial cavity is gradually divided into the right and the left atrium by the formation of the interatrial septum. When the right atrium first becomes recognizable, it is smaller than the left, but it expands at a more rapid rate than the left and eventually becomes the larger of the two. While the interatrial septum is developing, the single atrioventricular canal is divided by the endocardial cushions into two apertures, one leading to each ventricle. The interatrial, atrioventricular, and aortopulmonary septa all fuse to the endocardial cushions. Later in development, the walls of the atria are strengthened by muscular cords of various sizes.

**The interatrial septum.** The interatrial septum is found in all vertebrates above and including *Dipnoi* (lungfishes) as a feature accompanying the appearance of the pulmonary vein. During embryonic life, the septum remains partially open and thus allows a certain amount of blood from the right side of the heart to pass to the left side. In the chick embryo, the interatrial septum closes the interatrial canal completely and then is perforated immediately by multiple secondary openings that correspond functionally to the foramen ovale of the mammalian heart. Late in development, the primary septum is enlarged by secondary additions. The thicker added portion becomes the predominant part of the septum in the adult avian heart, except in passerine birds, which possess only the membranous primary septum (*Gasch*, 1888).

The interatrial septum is the first of the cardiac septa to start its development. In the chick, its initial appearance occurs after 48 to 55 hours' incubation (*Quiring*, 1933), when it can be seen as a small internal ridge on the cephalic wall of the atrium, somewhat to the right of the midline (Fig. 278-A). Its position corresponds to the external groove visible at this time (*Patten*, 1922). The ridge consists of proliferated endocardial cells (*Chang*, 1931*b*; *Quiring*, 1933); see Fig. 278-B. Before the sixty-fifth incubation hour, a preponderantly myocardial ridge, continuous with the endocardial proliferation, runs obliquely downward and to the left along the ventral wall of the atrium (*Chang*, 1931*b*), ending to the right of the atrioventricular canal (*Masius*, 1889).



The early septum has been regarded as a fold in the atrial wall, caused by constriction (*Bremer, 1928a*). If this view is correct, its subsequent growth may be either an actual protrusion of the wall into the heart or merely the result of expansion of the atrial cavities on either side of the folded septum. In the opinion of *Chang (1931b)*, the septum increases in size not through folding but through the growth of its endocardial portion, situated along its free margin, as well as through the upward stretching of its myocardial portion.



**Fig. 278.** Location and histological structure of the interatrial septum at the time of its appearance in the heart of the chick embryo incubated 50 to 55 hours. (Redrawn with modifications after *Quiring, 1933*.)

A, diagrammatic section of the heart at the level of the interatrial septum ( $\times 40$ ) as shown in the *Insert*; B, section of the interatrial septum ( $\times 250$ ).

$l_R$ ,  $l_L$ , right and left atrium; 2, interatrial septum; 3, sinus venosus; 4, bulbus; 5, dorsal mesocardium.

The comma-shaped interatrial septum rapidly grows down into the atrial cavity to the right of the mouth of the pulmonary vein (*Fig. 279*). By the end of the fourth incubation day, it is a crescentic partition extending into the lumen for about one third of the distance from the cephalodorsal atrial wall to the atrioventricular canal, and its dorsal and ventral extremities have fused with the dorsal and ventral endocardial cushions in the atrioventricular canal (*Quiring, 1933*). The decrease in the size of the interatrial opening causes blood to accumulate in the right atrium, so that the interatrial septum now bulges to the left, hiding the mouth of the pulmonary vein (*Chang, 1931b*). Simultaneously, the right atrium begins to enlarge (*Patten, 1922; Quiring, 1933*) with the result that the septum seems to move to the left (*Masius, 1889*).

In the chick, the interatrial septum finally occludes the interatrial canal in the third quarter of the fifth day (*Quiring, 1933*). At the same time, the dorsal and ventral endocardial cushions fuse with each other (*Masius,*



1889) and divide the atrioventricular canal into two openings. The septum now develops multiple perforations in a circumscribed area near the center (*Lindes*, 1865), where it is thinnest (Fig. 279-D; cf Fig. 280-B). These perforations seem to form at the weakest point in the septum as the result of the increasing pressure of blood in the right atrium, which is now as large as the left (*Quiring*, 1933).

In the European lapwing (*Vanellus vanellus*), the multiple perforations develop before the interatrial septum completes its downgrowth. The sep-

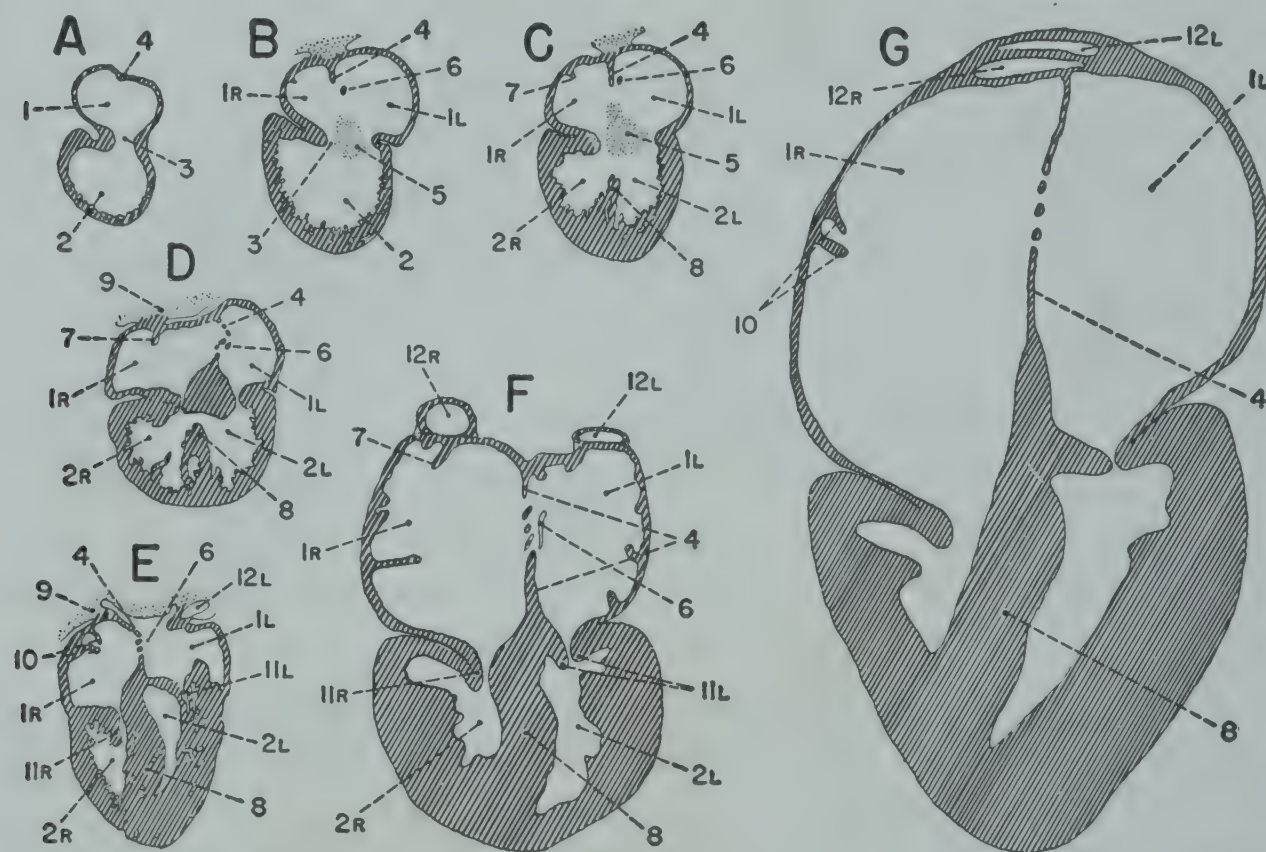


Fig. 279. Development of septa and valves in the heart of the chick embryo from the third to the eighteenth day of incubation, inclusive, as shown in semischematic frontal sections. (Redrawn with modifications after *Quiring*, 1933.)

A, at 65 hours; B, at 4 days; C, at 4.75 days; D, at 5 days; E, at 7 days; F, at 11 days; G, at 18 days. All  $\times 6$ .

1, undivided atrium; 1<sub>R</sub>, 1<sub>L</sub>, right and left atrium; 2<sub>R</sub>, 2<sub>L</sub>, right and left ventricle; 3, atrioventricular canal; 4, interatrial septum; 5, endocardial cushion of interatrial canal; 6, orifice of pulmonary vein; 7, septum spurium; 8, interventricular septum; 9, dorsal mesocardium; 10, valvulae venosae; 11<sub>R</sub>, 11<sub>L</sub>, right and left atrioventricular valve; 12<sub>R</sub>, 12<sub>L</sub>, right and left precaval vein.

tum reaches the level of the dorsal and ventral endocardial cushions and fuses with them before they fuse with each other; hence for a short time the atrioventricular canal is divided in two by the septum alone. During this period a large foramen is temporarily present near the lower edge of the septum (*Fuchs*, 1924).

As *Patten* (1925) pointed out, the small perforations in the chick's interatrial septum are so constituted that they can perform the functions of a valve. The openings are numerous and irregular (*Masius*, 1889), and the strands of tissue between them overlap each other (Fig. 280-C). When



the atria are expanded in diastole, blood from the sinus venosus flows into the right atrium toward the flaccid septum, which bulges to the left. The stretching of the septum opens the small foramina, and blood passes through them into the left atrium (Fig. 280-D). During systole, the contraction of the atrial walls and the closure of the sinoatrial valves act together to equalize the blood pressure on either side of the septum, which assumes its median position with foramina closed. It is possible that the contraction of the median dorsal muscular arch is in itself sufficient to

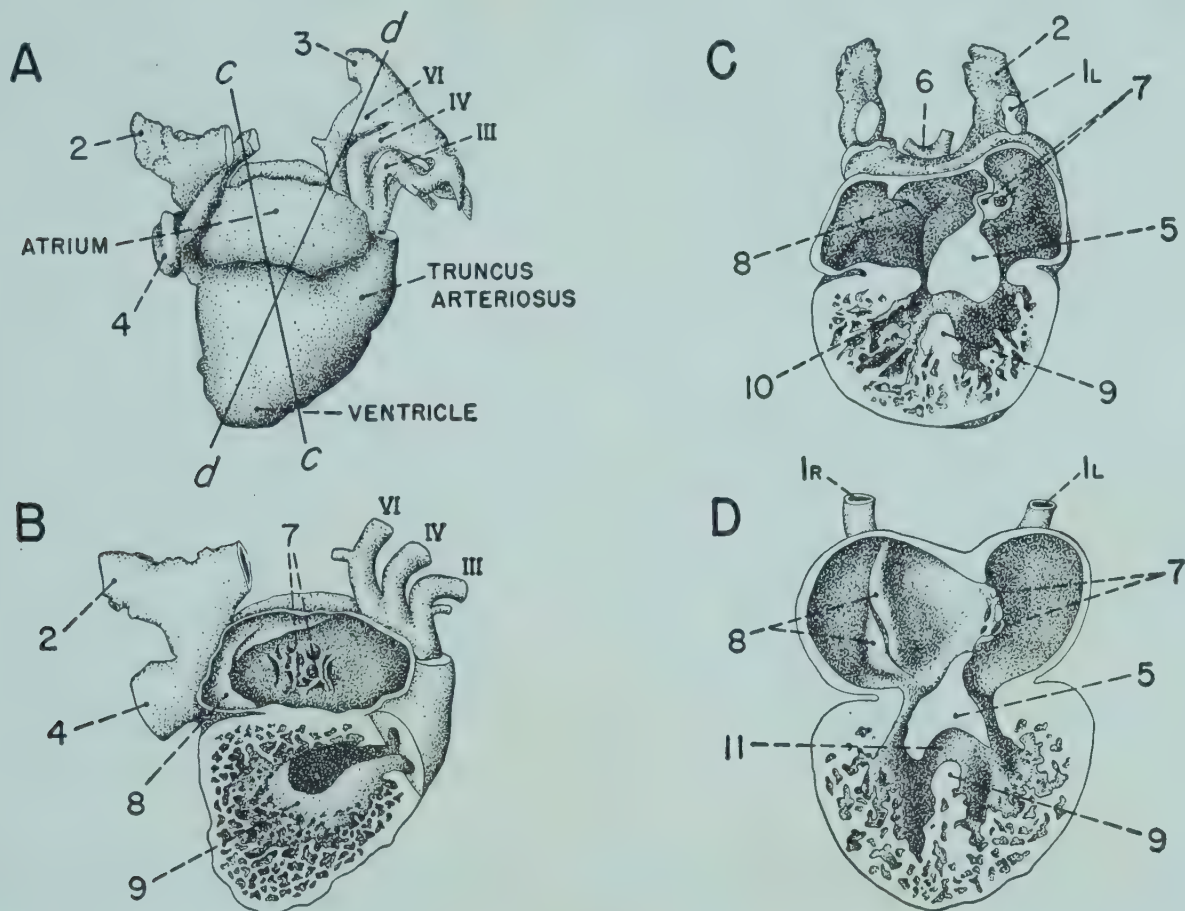


Fig. 280. The external and internal structure of the heart of the 5-day chick embryo. (Redrawn with modifications after Patten, 1925.)

A, dextral view of the exterior of the heart; B, dextral view of the heart after removal of part of the right wall; C, the interior of the caudal part of the heart, exposed by a section along the plane indicated by the line *cc* in A; D, the interior of the dorsocaudal half of a heart opened along the line *dd* in A. All  $\times 35$ .

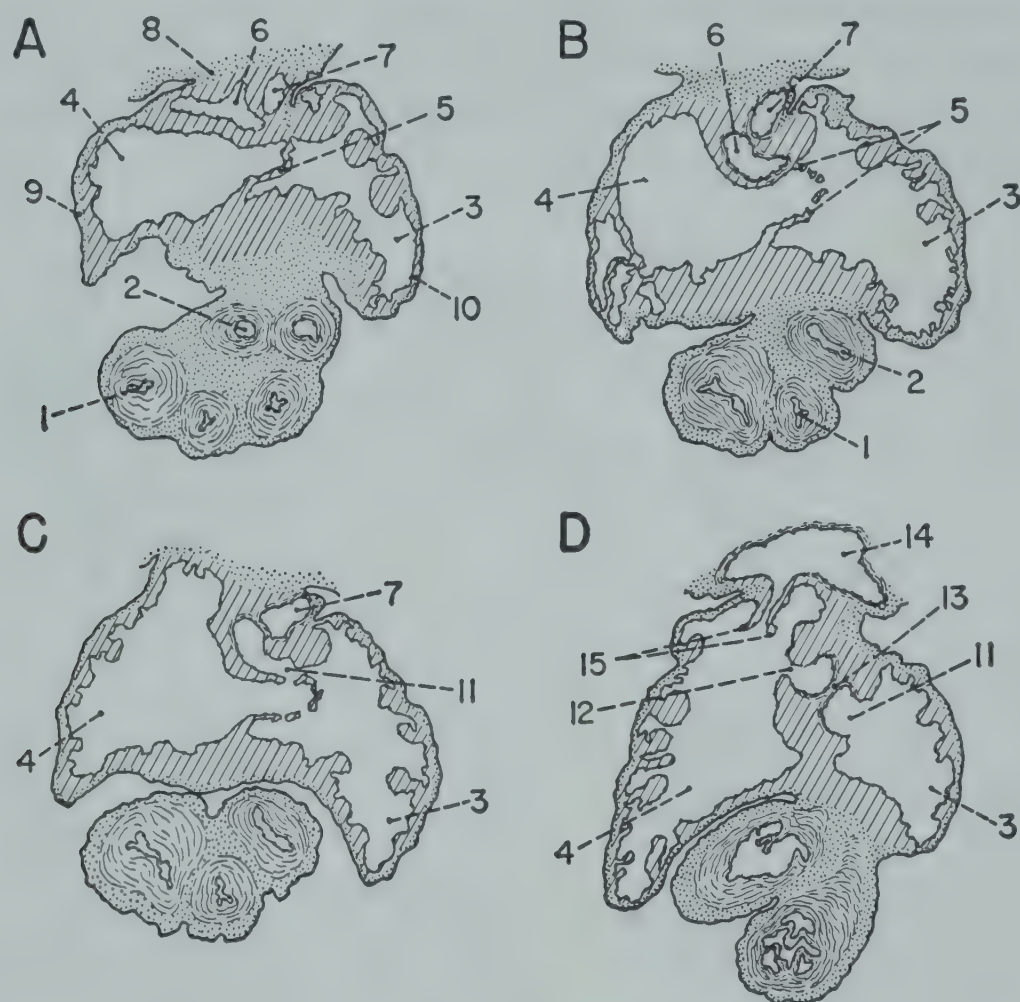
1<sub>R</sub>, 1<sub>L</sub>, right and left anterior cardinal vein; 2, posterior cardinal vein; 3, dorsal aorta; 4, inferior vena cava; 5, endocardial cushion of the atrioventricular canal; 6, pulmonary vein; 7, foramina in the interatrial septum; 8, valvulae venosae; 9, interventricular septum; 10, right atrioventricular canal; 11, interventricular foramen; III, IV, VI, aortic arches.

close the interatrial foramina (Quiring, 1933). When the lungs begin to function at the end of incubation, the pulmonary vein brings an increased volume of blood to the left atrium, and the posterior vena cava brings less blood from the allantois to the right atrium. The blood pressure on either side of the septum thus tends to be equalized permanently, and the obliteration of the foramina is favored (Patten, 1925). Their final closure usually occurs within a few days after hatching (Quiring, 1933).

Late stages in the development of the chick's interatrial septum were



studied by Quiring (1933). The atrial walls and the adjacent walls of the left precava and the pulmonary vein enter into a large secondary addition to the septum. After the seventh incubation day, the mouth of the pulmonary vein appears to shift to the right as the atria enlarge. This change causes the right wall of the pulmonary vein and the contiguous part of the



**Fig. 281.** Semischematic representations of cross sections through different levels of the atrial region of the 18-day chick embryo's heart, showing how the pulmonary vein and the left precaval vein enter the heart. (Redrawn with modifications after Quiring, 1933.)

A, section at a level above the mouths of the pulmonary and left precaval veins, which are embedded in the heart wall and the dorsal mesocardium; B, more caudal section, where the left precaval vein is dorsal to the pulmonary vein; C, still more caudal section, at the level of the pulmonary orifice; D, composite of two sections taken caudal to C, showing the left precaval orifice and the portion of the interatrial septum formed by the wall between the pulmonary and left precaval veins; the sinus venosus does not extend as far as this level but is included to show its position relative to the other chambers. All  $\times 7$ .

1, aorta; 2, pulmonary artery; 3, left atrium; 4, right atrium; 5, interatrial septum; 6, pulmonary vein; 7, left precaval vein; 8, dorsal mesocardium; 9, epicardium; 10, endocardium; 11, orifice of pulmonary vein; 12, orifice of left precaval vein; 13, wall between pulmonary and left precaval veins; 14, sinus venosus; 15, valvulae venosae.

wall of the right atrium to be added to the septum. By the eleventh day, the pulmonary orifice is surrounded by a crescentic fold continuous with the septum and derived both from the wall of the vein and from that part of the left atrial wall lying immediately over the vein (cf. Fig. 282-A). This fold gradually extends farther into the atrial cavity as the pulmonary vein



is drawn into the left atrium. During the final days of incubation, the left margin of the pulmonary orifice projects as a flap (cf. Fig. 282-B) similar to the valvelike structure which closes the pulmonary orifice in the adult heart (cf. Fig. 282-C) when the auricles contract (*Gasch*, 1888).

The left precaval vein contributes to the lower portion of the secondary part of the septum. At the 18-day stage (Fig. 281), the terminal portion, or root, of the left precaval vein is found deeply embedded in the left wall of the right atrium, at a lower level than the terminal portion of the pulmonary vein (cf. Fig. 282-B). A large flap of tissue derived from the atrial wall hangs over the precaval orifice. The right side of the lower part of the secondary addition to the septum is thus formed of the left precava and the wall of the right atrium. The primary portion of the interatrial septum migrates to the left and becomes relatively reduced in size as the secondary portion enlarges. Over half of the definitive septum is formed of the secondary addition, which bulges prominently to the right (cf. Fig. 282-C).

*The "muscular arches" of the atrial walls.* A prominent feature of the avian heart is the presence of muscular cords in the dorsal walls of the atria (cf. Fig. 282-C). One such cord, designated by *Quiring* (1933) as the median dorsal arch, lies above the line of attachment of the interatrial septum. A right and a left lateral arch extend transversely across the roof of the right and left atrium, respectively, and these lateral arches branch into smaller muscular "ribs," the *musculi pectinati*, which in turn are fused with a muscular band forming the inferior limits of the atria. In the right atrium, one of the ribs is anastomosed with the left sinoatrial valve and may act as a tensor for this valve. During atrial systole, the contraction of the entire muscular system tends to obliterate the atrial cavities, close the left precaval and pulmonary orifices, and compress the primary portion of the interatrial septum.

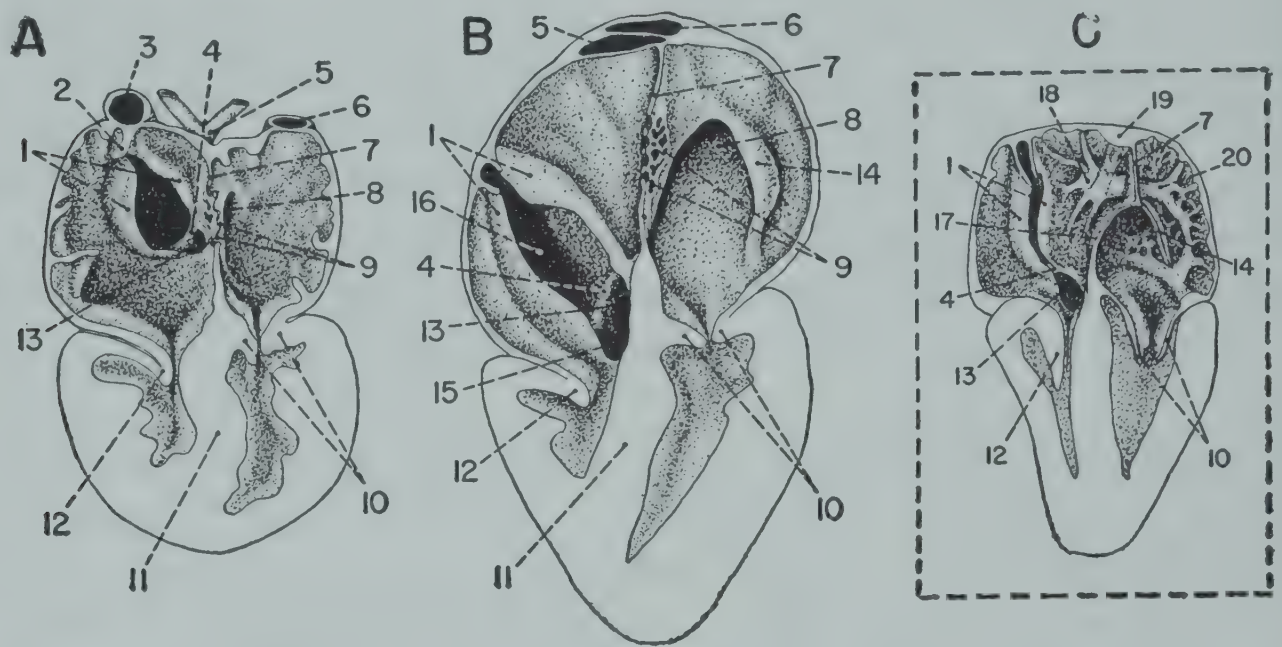
In the 5-day chick embryo, the lateral atrial walls exhibit multiple out-pocketings, as if a thin sheet of circularly disposed muscle had been separated into parallel bundles as the atria expanded (*Bremer*, 1928a). The initial development of the muscular cords is indicated by thickenings of the dorsal atrial walls; differentiation into definite cords follows during the seventh day. In the beginning, the muscular structures evolve more rapidly in the right atrium than in the left (*Quiring*, 1933).

Between the seventh and eleventh incubation days, the lateral muscular arches develop rapidly. At the end of incubation, they have become much more prominent than the median dorsal arch (*Quiring*, 1933).

*The valvulae venosae and the sinus septum.* In adult birds, there is considerable variation in the mode of entry of the large veins into the right atrium. According to *Gasch* (1888), the sinus venosus does not exist as a separate entity in such birds as *Ardea*, *Grus*, *Otis*, *Tetrao*, *Columba*,



*Cuculus*, and the “*Raptatores*” and “*Natatores*,” in all of which the inferior vena cava and the right and left superior venae cavae open directly into the right atrium. In other birds, however, the retention of the sinus venosus is indicated by the fact that the inferior vena cava shares a common orifice with either the right precaval vein (in *Cornix* and the *Fringillae*) or both precaval veins (in *Casuarius* and *Struthio*). The coronary vein usually opens directly into the left precaval vein or behind its valves and may have two or three orifices.



**Fig. 282.** Late stages in the development of the valvulae venosae and the interatrial septum in the heart of the chick embryo, as seen from the ventral side; a similar view of the adult chicken heart is shown for comparison. (Redrawn with modifications after Quiring, 1933.)

A, at 11 days ( $\times 7$ ); B, at 18 days ( $\times 7$ ); C, adult ( $\times 0.8$ ).

1, sinoatrial valves; 2, septum spurium; 3, right precaval vein; 4, sinus septum; 5, pulmonary vein; 6, left precaval vein; 7, interatrial septum; 8, orifice of pulmonary vein; 9, perforations in interatrial septum; 10, left atrioventricular valves; 11, interventricular septum; 12, right atrioventricular valve; 13, orifice of left precaval vein; 14, valve at orifice of pulmonary vein; 15, orifice of coronary vein; 16, sinus venosus; 17, secondary part of interatrial septum; 18, right lateral muscular arch; 19, median muscular arch; 20, left lateral muscular arch.

In the chicken, the postcaval vein and the right precava open together into the sinus venosus, whose slitlike entrance into the right atrium is well demarcated by a pair of valves, the valvulae venosae, or sinoatrial valves. The sinus septum separates the mouths of the left precava and coronary vein from the common orifice of the other veins (cf. Fig. 282-C).

According to the account given by Chang (1931*b*), the right sinoatrial valve forms first. In the 75-hour chick embryo, the right atrium has expanded to the right, and its expansion has produced a fold of the heart wall at the junction of the right atrium and the sinus (cf. Fig. 273-D<sub>2</sub>; cf. Fig. 274-D). This fold constitutes the early right valve (Masius, 1889).

On its left side, the sinus is temporarily delimited from the atrium at the 62- to 65-hour stage by the early interatrial septum, which, at this time,



consists only of a small endocardial ridge. By the 96-hour stage, the left sinoatrial valve has formed, presumably from the wall of the right atrium in much the same manner as the right valve. At its lower end, the left valve is confluent with the interatrial septum. The sinoatrial opening is already slitlike.

The valvulae venosae meet at the upper end of the sinoatrial opening and are continuous with a ridge on the roof of the right atrium (*Masius*, 1889) known as the septum spurium or crista terminalis (cf. Fig. 280-C and D). This ridge may be formed by the fusion of the two valves, but it is possible that the ventral wall of the right precaval vein is involved, since this vein lies directly dorsal to the junction of the valvulae venosae (*Quiring*, 1933).

By the chick's seventh incubation day, the lower part of the left sinoatrial valve has become reduced to a very slight ridge where it bounds one side of the left precaval orifice. On the other side of the left precaval orifice—that is, the side which lies within the cavity of the sinus—there is now another ridge, the early sinus septum. This septum has become quite prominent by the 11-day stage (Fig. 282-A). From its point of origin on the sinus surface of the left valve, it extends diagonally downward and to the right, separating the upper part of the sinus cavity (which receives the right precaval vein and the inferior vena cava) from the left precaval orifice and the mouth of the coronary vein, to the right of and slightly below the precaval orifice. The lower part of the right sinoatrial valve thus acts as the valve for the coronary vein. Later, the sinus septum stretches entirely across the sinus from the left to the right valve (Fig. 282-B and C).

**The Endocardial Cushions of the Atrioventricular Canal.** In the opening leading from the atrial to the ventricular cavity, two massive endocardial proliferations, one dorsal and one ventral, grow out into the lumen of the heart tube between the two blood streams. Two smaller lateral proliferations also develop. These four proliferations are the endocardial cushions. The dorsal and ventral cushions meet and fuse in the center of the atrioventricular canal and thus divide the latter in two.

The division of the atrioventricular canal is the first step in the division of the primitive ventricle and the bulbotruncus, for the dorsal and ventral endocardial cushions are part of the longitudinal partition that divides the heart. Because the heart is bent and rotated, however, the cushions seem to constitute a focal point toward which all the cardiac septa grow.

In the 65- to 84-hour chick embryo, the dorsal and ventral endocardial cushions are recognizable as thick cellular structures (*Chang*, 1931b; *Patten, Kramer, and Barry*, 1948). They each develop a right and left protuberance. As they grow centripetally into the atrioventricular canal, their bases widen transversely, keeping pace with the growth of the atrial region. The atrioventricular canal is thus transformed into a roughly H-shaped



opening the transverse portion of which is long and the two lateral portions short (*Masius*, 1889). Fusion of the dorsal and ventral cushions eventually obliterates the transverse fissure but leaves the two lateral slits open; these are the definitive atrioventricular canals. According to Bremer (1928a), fusion has occurred by the middle of the fifth incubation day, shortly before the interatrial septum is complete. Other workers (*Masius*, 1889; *Quiring*, 1933) have reported that the cushions fuse with each other at the moment when they complete their fusion with the interatrial septum, that is, at the end of the fifth day (*Quiring*, 1933). The right side of the dorsal endocardial cushion fuses with the dorsal portion of the interventricular septum. The right side of the ventral cushion fuses with an endocardial ridge within the right side of the mouth of the bulbus, and the sheet of fused tissue, by growing toward the apex of the heart, separates the mouth of the bulbus from the right atrioventricular canal (*Hochstetter*, 1906). The left side of the fused cushions becomes part of the left atrioventricular valve.

**The Atrioventricular Valves.** The left atrioventricular valve of the avian heart is structurally similar to the corresponding mammalian valve in that it is usually formed of two cusps, one lateral and one central. Occasionally, however, it is tricuspid in birds. The fibrous chordae tendineae extend from the lower surfaces of the valve cusps to the three papillary muscles of the left ventricle and prevent the valve from opening into the left auricle during ventricular contraction. The right atrioventricular valve consists of a single lateral leaf in the form of a thick muscular flap in which is embedded fibrous tissue containing a sheet of Purkinje fibers (part of the conduction system of the heart). The right valve originates at the right side of the base of the pulmonary artery, where it is held by a small trabecular muscle (there are no chordae tendineae in the right ventricle). From this point the valve extends dorsad and somewhat caudad around the right ventricular wall to the junction of the latter with the interventricular septum. When the right ventricle contracts, the free edge of the valve leaf is forced upward against the bulging interventricular septum and closes the slitlike atrioventricular ostium; during diastole, the valve drops downward into the ventricle and thus opens the atrioventricular ostium.

At the chick's fiftieth hour of incubation, the atrioventricular canal closes when the heart contracts because the cardiac jelly between the endocardium and the myocardium of the canal accumulates in "mounds" that occlude the canal. Somewhat later, valvular action is exerted by the apposition of the endocardial cushions, which are formed by the invasion of cells into the cardiac jelly (*Patten, Kramer, and Barry*, 1948). However, only portions of the endocardial cushions enter into the formation of the definitive atrioventricular valves.



The central or septal cusp of the left atrioventricular valve is derived from the left half of the fused dorsal and ventral endocardial cushions, that is, from the portion that is not involved in fusion with the interventricular septum. The lateral cusp of the left valve is formed in part by the reflected wall of the auricular canal and in part by the lateral endocardial cushion, which arises soon after the dorsal and ventral cushions appear but which never attains a comparable size. The papillary muscles of the left side originate from muscular trabeculae joining the folded atrial wall with the wall of the ventricle (*Hochstetter, 1906*).

The right atrioventricular valve, which is largely muscular, is formed by the inflection of ventricular and auricular muscle, the auricular component forming a thin inner (or upper) layer (*Davies, 1930a*). Part of the auricular component may represent a secondary ingrowth of muscle tissue from the wall of the auricular canal. An insignificant endocardial component is derived from the right lateral endocardial cushion (*Hochstetter, 1906*).

**The Ventricles and the Interventricular Septum.** The development of the ventricles begins during the chick's third incubation day as an expansion of the bottom of the ventricular curve of the heart tube caused by the centrifugal force of the blood stream. The expansion is not a simple enlargement but consists of multiple outpocketings of the ventricular wall, as if the endocardium had bulged between circular bundles of muscle (*Bremer, 1928a*). The muscular bundles separating the small aneurysmal pouches are the first anlagen of the trabeculae carneae of the ventricles. These are the largely tendinous but partly muscular bundles that form a network of varying prominence in the ventricles of adult birds of different species. For example, they are said (*Gadow, 1891*) to be well developed in the cassowary (*Casuarius* sp.) and the rhea (*Rhea americana*), but to be only insignificant threads in the ostrich (*Struthio camelus*).

The first ventricular expansion to form represents the left ventricle; it lies in the path of the blood stream passing through the left atrioventricular canal (*Bremer, 1928a*). During the chick's fourth incubation day, the stream from the right atrioventricular canal forces out the ventricular wall on the right side. The resulting expansion, which also exhibits multiple pouches, is the right ventricle. The two ventricles are separated by a portion of the trabecular wall that has remained relatively unaffected by the force of the blood streams (*Chang, 1932*); this is the early interventricular septum. At the end of the fourth day, the septum consists of loose trabeculae little different from those lining the ventricular walls, and is but little higher. Its position corresponds to that of the interventricular furrow on the external surface of the heart. The interventricular furrow is continuous cranially with the bulboauricular furrow, and the interventricular septum is continuous with the internal bulboauricular ridge (*Hochstetter, 1906*).



By the middle of the fifth day, the right ventricle includes the portion of the bulbus that heretofore formed the sharp lateral curve of the transverse arm of the bulbar spiral. This proximal part of the bulbus is drawn down into the heart (*Hochstetter, 1906*) as the bulbar spiral begins to loosen (*Bremer, 1928a*). The incorporation of the proximal bulbus into the right ventricle is accompanied by an undermining or excavation of its wall, which for a time consists of an internal and an external lamella connected by trabeculae (*Hochstetter, 1906*). A similar process has been observed in the lapwing (*Vanellus vanellus*) embryo (*Fuchs, 1924*).

The interventricular septum grows higher (cf. Fig. 279-C and D) and soon extends around the apex of the heart from the dorsal to the ventral wall of the ventricular cavity (cf. Fig. 280-B). Its free border is a curve with concavity directed cranially, that is, toward the base of the heart. The septum is inclined toward the right, however, and the free border is directed not toward the center of the endocardial cushions, but toward their right side (cf. Fig. 280-C and D). The dorsal portion of the septum, which grows more rapidly than the ventral portion, becomes continuous with the right side of the endocardial cushions shortly after the interatrial septum becomes complete (*Hochstetter, 1906*), that is, at the end of the chick's fifth incubation day (*Quiring, 1933*).

The right ventricle meantime increases in size, and the entire ventricular portion of the heart continues to rotate to the left, bringing the right ventricle and the bulbus to a still more ventral position. This rotation, which appears to be due to the growth of the right ventricle (*Masius, 1889*), may be attributed also to pressure from the growing right atrium and to the continued unwinding of the bulbar spiral (*Bremer, 1928a*).

The loose trabecular structure of the early interventricular septum becomes increasingly dense as the septum grows higher and thicker (*Hochstetter, 1906*). At the 5-day stage, it is massive and muscular (cf. Fig. 280-C and D), and its endocardial layer is especially thick on the right side (*Chang, 1932*); a day later it is a complete partition between the ventricles except for the small interventricular foramen in its upper part. Because of the great thickness of the septum, the foramen is actually a channel, tapering in diameter from left to right (*Tonge, 1869*). The further development of the interventricular septum is inextricably interrelated with that of the aortopulmonary septum.

The ventricular walls gradually become thicker as incubation proceeds (cf. Fig. 279). From the middle of the chick's eighth day of development to the end of incubation, the wall of the left ventricle increases from about 100 microns in thickness to about 1100 microns. The wall of the right ventricle is consistently about half as thick as that of the left ventricle (*Hughes, 1943*). The ventricular walls also become more compact and muscular as the trabeculae grow more massive. The intertrabecular spaces



diminish greatly in size and number between the twelfth and the sixteenth incubation day, and only a few trabeculae persist at the latter time (*Schockaert, 1909*).

**The Aortopulmonary Septum and the Semilunar Valves.** The distal portion of the bulbotruncus, which is not incorporated into the heart, is divided into two arterial trunks by the aortopulmonary septum. The arterial trunks, of course, are the proximal continuations of the aorta and the pulmonary artery. The union of the aortopulmonary and interventricular septa leaves the aorta in continuity with the left ventricle and the pulmonary artery in continuity with the right ventricle. But for the existence of the multiple interatrial foramina, the systemic and pulmonic circulations would then be completely separated.

Essentially, the division of the bulbotruncus is achieved in the same manner as the division of the atrioventricular canal, that is, by the proliferation and eventual confluence of two opposing endocardial ridges formed between the two blood streams of the heart. The fundamental simplicity of the process is obscured somewhat by the spiral course of these blood streams through the bulbotruncus and by the gradual transfer of the proximal part of the bulbotruncus to the ventricular portion of the heart. In addition, septum formation is complicated by the appearance of supplementary endocardial thickenings which give rise to the two semilunar valves at the roots of the aorta and the pulmonary artery, respectively. In their definitive state, these valves each consist of three pouchlike cusps (ventral, central, and lateral) directed away from the heart and preventing the backflow of blood. In the very early stages of heart development in both the chicken (*Patten, Kramer, and Barry, 1948*) and the duck, *Anas platyrhynchos* (*Boerner-Patzelt, 1931*), the function of a bulboventricular valve is performed by "endocardial mounds" at the junction of the primitive ventricle and the bulbus. These are similar to the atrioventricular "mounds" and open when the latter close (*Patten, Kramer, and Barry, 1948*).

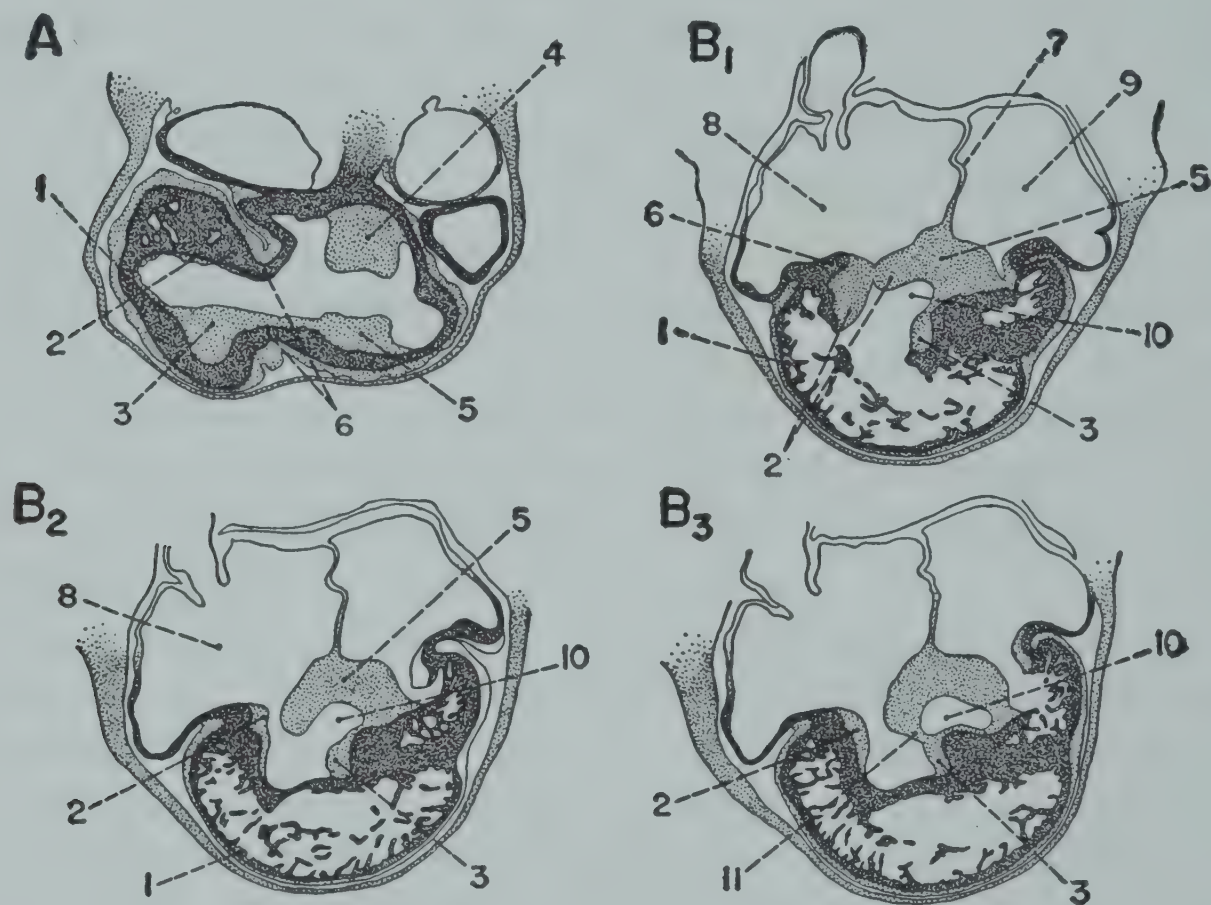
The bulbar endocardial ridges that take part in septum formation develop independently in the distal and proximal portions of the bulbotruncus, above and below the level where the two blood streams become confluent. Both pairs of ridges (distal and proximal) outline the course of the blood stream which enters the bulbar region from the right side of the ventricular cavity and then winds around the stream entering from the left side; its course is a spiral turn from right to left and from ventral to dorsal. The position of each ridge, therefore, is reversed as it passes from the distal to the proximal end of the bulbotruncus, the ridge arising on the left side terminating on the right side, and vice versa (cf. Fig. 277).

At the distal end of the truncus, the ridges are continuous with the ends of the almost horizontal arterial wall separating the fourth and the



sixth aortic arches. The fusion of the two opposing ridges, progressing heartward, brings about an ingrowth of this arterial wall as the aortopulmonary septum. The distal separation of the arterial trunks begins in this manner in the 4.5-day chick embryo (Tonge, 1869).

The proximal bulbar ridges can be seen in the embryo of the lapwing (*Vanellus vanellus*) at the 38-somite stage (Fuchs, 1924). Their respective dorsal and ventral positions at the 51-somite stage can be seen in Fig.



**Fig. 283.** Semischematic representations of horizontal sections through the atrial and bulbar region of the heart of the European lapwing (*Vanellus vanellus*) embryo, showing two stages in the development of the aortopulmonary and interventricular septa. (Redrawn with modifications after Fuchs, 1924.)

A, at the 51-somite stage; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, sections taken successively closer to the apex of the heart at a stage corresponding to that of about 5.5 days' incubation in the chick.

1, wall of bulbus; 2, dorsal (right) proximal bulbar ridge; 3, ventral (left) proximal bulbar ridge; 4, dorsal endocardial cushion; 5, ventral endocardial cushion; 6, bulboatrial ridge (or groove); 7, interatrial septum; 8, right atrium; 9, left atrium; 10, conus arteriosus (interventricular foramen); 11, extension of dorsal proximal bulbar ridge growing along upper edge of interventricular septum and uniting both proximal bulbar ridges.

283-A. During the latter half of the chick's fifth incubation day the bulbus rotates from right to left (Masius, 1889; Langer, 1894; Hochstetter, 1906), so that it lies closer to the midline and ventral instead of lateral to the right atrium. This change is apparent in Fig. 283-B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, which show sections of the lapwing (*Vanellus vanellus*) embryo's heart at a stage equivalent to that of 5.5 days in the chick. The bulbar ridge that was formerly ventral in position is now on the left side of the bulbar cavity. It



is continuous caudally (toward the apex of the heart) with the ventral part of the interventricular septum. The right bulbar ridge (formerly dorsal) is now in such a position that it can become continuous with the right lateral endocardial cushion and with the right side of the ventral endocardial cushion (*Masius, 1889; Hochstetter, 1906*). A bridge is thus formed across the opening of the bulbus into the atrioventricular canal, and the dorsal wall of the aorta is prolonged downward (*Hochstetter, 1906*). It is at this stage (*Tonge, 1869*) that the left proximal bulbar ridge becomes continuous with the dorsal distal ridge.

At the end of the sixth day, the interventricular septum has increased greatly in thickness, and the aperture in it has thus been transformed into a funnel-shaped channel with its smaller right end directed toward the mouth of the aorta (*Tonge, 1869*). This channel is the primitive conus arteriosus. A vertical plane through the interventricular septum crosses a vertical plane through the lower border of the aortopulmonary septum almost at right angles. At this time, the root of the pulmonic trunk is ventral to the root of the aorta and somewhat to its left.

The continuity of the aortopulmonary and interventricular septa becomes complete at the end of the seventh day (*Bremer, 1928a*) or during the eighth day (*Tonge, 1869*). The proximal bulbar ridges continue to fuse together and thus to prolong the aortopulmonary septum downward; and the aortopulmonary septum fuses with the ventral part of the right side of the interventricular septum (the portion bordering the interventricular foramen). In this way, the communication between the aortic and pulmonic trunks is closed and the right and left ventricles are separated. The interventricular foramen remains in continuity with the lumen of the aorta, which therefore receives blood from the left ventricle (*Tonge, 1869; Masius, 1889; Hochstetter, 1906*). The pulmonic trunk receives blood from the right ventricle, and its root is now somewhat to the right of the aortic root.

The external separation of the aorta and the pulmonic trunk starts at the end of the seventh day. The ingrowth of the outer walls of the truncus arteriosus external to the aortopulmonary septum has progressed down to the level of the semilunar valves by the middle of the eighth day and down to the bases of the ventricles by the thirteenth day (*Tonge, 1869*).

The development of the semilunar valves begins when the distal division of the bulbotruncus starts (*Tonge, 1869*). The anlagen of the ventral cusps of the valves are the first to form, appearing as two elongated projections, somewhat pyramidal in section and separated by a groove. They are situated far from the heart and only a short distance below the early aortopulmonary septum. The left distal bulbar ridge passes between them. By the time fusion of the two opposed distal ridges reaches the level of the valve rudiments, the latter have grown larger. The primordia of the central



aortic and pulmonary valve cusps now appear, projecting from each side of the aortopulmonary septum (*Hochstetter, 1906*). The ridges representing the outer valve cusps are the last to form (*Hochstetter, 1906*), but they also arise before the end of the fifth incubation day (*Tonge, 1869*).

At the 6-day stage, the semilunar valves are still solid, but their upper surfaces are now beginning to become concave. Their cusps have grown so large that they prevent the reflux of blood (cf. Fig. 277-E) and give the lumen of each arterial trunk the shape, in cross section, of a three-rayed star (*Tonge, 1869*).

By the beginning of the seventh day, the proximal ends of the semilunar valves (heretofore a considerable distance from the bulboventricular junction) have been brought close to the bases of the ventricles by the continued incorporation of the proximal bulbus into the right ventricle. The excavation of the semilunar valves to form pouches starts at the end of the seventh day, when the rudiments of the ventral and inner (central) cusps of both aortic and pulmonary valves are just beginning to be hollowed out. The lateral valves start to undergo this process at the end of the eighth day (*Tonge, 1869*).

### The Innervation of the Heart

The heart is supplied with a ganglionated plexus of nerves which ramify on the surface of the auricles and fan out over the ventricles from the coronary sulci. From this superficial plexus, fibers reach the sinoauricular and auriculoventricular nodes. The fibers forming the cardiac plexus are both sympathetic and parasympathetic. The sympathetic fibers, which act to accelerate the heartbeat, arise in the intermediolateral cell column of the spinal cord and run to the superior and inferior cervical sympathetic ganglia, whence postganglionic neurones proceed to the cardiac plexus in association with fibers of the vagus nerve. Some of the sympathetic fibers are afferent. The parasympathetic fibers, which decrease heart frequency, arise in the dorsal motor nucleus of the vagus nerve and proceed in branches of this nerve to ganglia of the cardiac plexus (especially those in the auriculoventricular furrow), where they synapse with postganglionic neurones. Fibers from the right and the left vagus respectively predominate in supplying the posterior and the anterior surface of the heart. The vagus trunk also contains the afferent fibers of the depressor nerve, stimulation of which reduces arterial blood pressure. Near the heart, the vagus gives off the recurrent nerve, which sends rami to the pulmonary artery and the heart. The ramus to the heart is the depressor nerve.

The cardiac plexus was regarded by His, Jr. (1893), as two plexuses, the bulbar plexus and the atrial plexus. The bulbar plexus is found around and between the aortic and pulmonic trunks and sends fibers into the atrioventricular furrow and the coronary sulci, thence to the ventricles. It



consists of elements of the cardiac vagal branches and of sympathetic elements from the cervical ganglia. The atrial plexus covers the upper and posterior surfaces of the sinus venosus and the venae cavae. It is composed of elements of the cardiac vagal branches and elements from the bronchial plexus and is connected with the bulbar plexus on the right side only.

The innervation of the chick embryo's heart apparently begins at the end of the third incubation day (*Szepesenwol and Bron, 1935*), rather than during the fifth day, as formerly thought (*Kuntz, 1910; Abel, 1912*). In the 68-hour chick (or 90-hour duck, *Anas platyrhynchos*), fibers originating from the thoracic ganglion of the vagus nerve attain the distal part of the truncus arteriosus by way of the aortic arches. No sympathetic elements are as yet present. It is possible that these first vagal fibers are sensory rather than motor, for they end in terminal buttons in direct contact with the vascular endothelium (*Szepesenwol and Bron, 1935*). Sympathetic elements begin to accompany the vagus fibers after the 80- to 84-hour stage (or, in the duck, *Anas platyrhynchos*, the 98-hour stage), when an anastomosis is formed between the thoracic ganglion of the vagus and the superior cervical sympathetic ganglion (*Szepesenwol and Bron, 1935*). Thus *Abel (1912)* observed vagal fibers intermingled with sympathetic cells in the vicinity of the truncus arteriosus of the 108- to 112-hour chick, although it was her opinion that the sympathetic cells were migrating in advance of the vagal fibers. She also found vagal and sympathetic elements in the septum of the truncus of the 5- to 6-day chick embryo.

The branch of the vagus proceeding to the auricular part of the heart reaches its destination in the chick embryo at the end of the fourth day and about a day later in the duck (*Anas platyrhynchos*) embryo (*Szepesenwol and Bron, 1935*). *Abel (1912)* remarked that nerve cells and fibers grow out from the pulmonary plexus into the dorsal mesocardium during the chick's sixth incubation day; she found them forming a definite plexus around the venous openings into the auricle at the end of this day, and entering the auricular wall at the end of the seventh day.

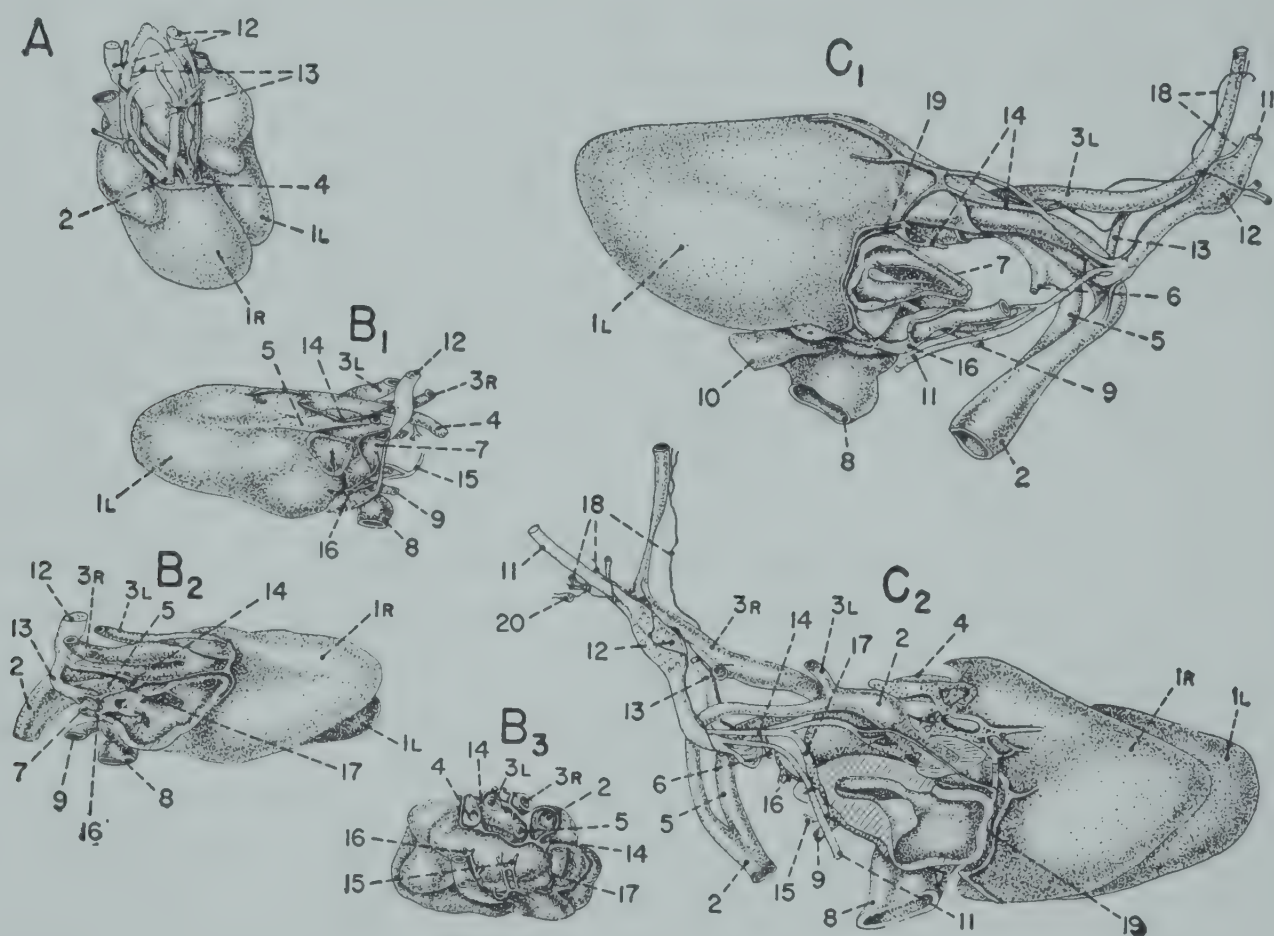
The heart nerves of the 6-day chick embryo (Fig. 284-A) were described similarly by *His, Jr. (1893)*, and *Abel (1912)*. The cardiac branches of the right and left vagi both course in the septum trunci arteriosi, and each ends in a small ganglion. Each vagus branch gives off the recurrent nerve at the level of the third aortic arch; this branch ends in a ganglion near the root of the subclavian artery. In the specimen shown in Fig. 284-A, the recurrent nerve on the left side has given off a small ramus that courses in the septum of the truncus parallel to the other two cardiac branches of the vagus.

In the 7-day embryo (*Abel, 1912*), the bulbar plexus may be seen taking origin at the point of juncture of the ventricular wall with the aorta and pulmonary artery. This plexus is a continuation of a plexus in the septum



of the truncus, which is in turn derived from the nerves previously present in the septum. Nerve fibers are now found in the posterior interventricular groove just below the auriculoventricular groove; these may be traced upward to the plexus around the venous openings into the auricle.

At the 8-day stage (*His, Jr., 1893; Abel, 1912*), there is a complex network of cells and fibers at the junction of the large arterial trunks with the ventricle (Fig. 284-*B*<sub>1</sub>, *B*<sub>2</sub>, and *B*<sub>3</sub>). Cells and fibers proceed from this plexus toward the auriculoventricular groove. Soon fibers from both the



**Fig. 284.** The development of the cardiac ganglia and plexuses in the chick embryo. (Redrawn with modifications after *His, Jr., 1893*.)

*A*, at 6 days, ventral aspect; *B*<sub>1</sub>, *B*<sub>2</sub>, *B*<sub>3</sub>, at 8 days, left side, right side, and dorsal aspect, respectively; *C*<sub>1</sub>, *C*<sub>2</sub>, at 10 days, left and right side, respectively. All  $\times 8$ .

1<sub>R</sub>, 1<sub>L</sub>, right and left ventricle; 2, aorta; 3<sub>R</sub>, 3<sub>L</sub>, right and left carotid artery; 4, pulmonary trunk; 5, ductus arteriosus; 6, pulmonary artery; 7, superior vena cava; 8, inferior vena cava; 9, pulmonary vein; 10, hepatic vein; 11, vagus nerve; 12, nodose ganglion of vagus nerve; 13, recurrent nerve; 14, bulbus nerve; 15, ramus from the pulmonary plexus; 16, atrial nerve and plexus; 17, connection between the bulbus nerve and the atrial plexus; 18, sympathetic rami; 19, coronary nerve; 20, first thoracic sympathetic ganglion.

right and left vagal branches form a plexus in the external auriculoventricular groove. This plexus is directly connected with the fibers that pass down the posterior interventricular wall and from which offshoots are now entering the upper part of the ventricular wall. The plexus in the auricular wall is extending down toward the auriculoventricular groove.

By the end of the tenth day (*His, Jr., 1893*), the coronary nerves have arisen from the bulbar plexus (Fig. 284-*C*<sub>1</sub> and *C*<sub>2</sub>). The right nerve originates anterior to the arterial trunks and the left nerve posterior to



them. These nerves lie in the coronary sulci, parallel to the coronary arteries, but do not yet reach the ventricular surface. According to Abel (1912), the interatrial septum still contains no neural elements at this time; this observation is irreconcilable with that of Kuntz (1910), who traced cells from the vagi into the interatrial septum at the 5-day stage.

### The Histogenesis of Cardiac Tissue

The avian heart is composed of three tissue layers, the epicardium, the myocardium, and the endocardium. The myocardium or muscular layer is the thickest and makes up the largest part of the heart; it is especially thick in the ventricles. The epicardium covers the myocardium externally and the endocardium lines it on its internal surface.

The histogenesis of cardiac tissue may be considered as starting with the differentiation of endothelial cells from the splanchnic mesoderm. These cells comprise the primitive endocardium. Somewhat later, but still very early in development, the cells of the outer heart tube (the epimyocardial mantle) begin to differentiate as typical myocardial elements. The epicardium is also derived from the outer heart tube.

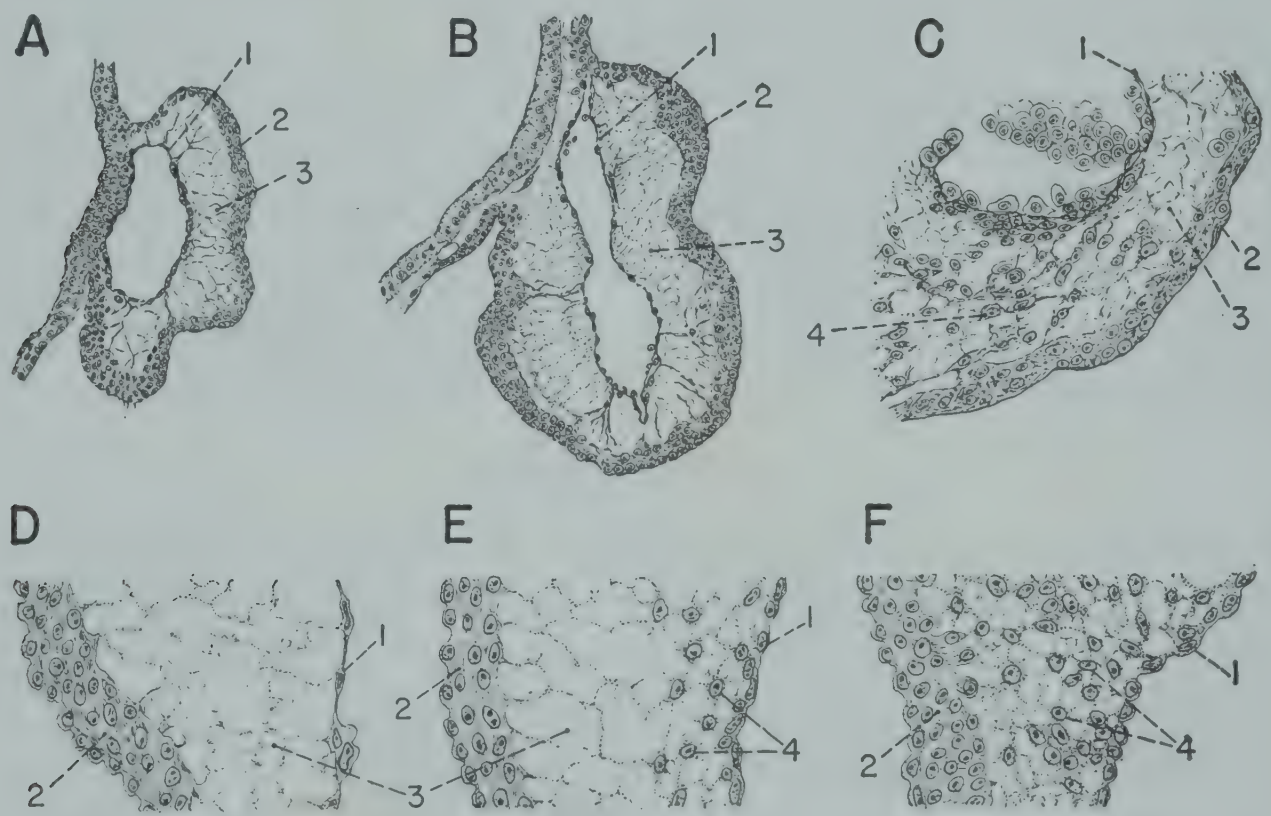
#### *The Endocardium*

The endocardium is essentially a type of connective tissue containing elastic fibers. Its free surface, facing on the heart cavities, is covered with a layer of flat endothelium. In its deeper portion it is traversed by a network of capillaries and also by the specialized muscle fibers (Purkinje fibers) which play an important role in the conduction of stimuli. The endocardium varies in thickness in different regions of the heart and is thinnest where it is subject to the greatest friction from the force of the blood stream. It is thinner in the ventricles, therefore, than in the atria, and it is thicker in the left atrium than in the right (*Chang, 1932*). As previously noted, it becomes enormously thickened between the two blood streams that pass through the heart and thus gives rise to the endocardial cushions and other septal and valvular tissue.

The development of most of the thick endocardial tissue proceeds at the expense of the cardiac jelly, which is confined to the portion of the heart derived from the original double-walled tube, that is, the bulbotruncus and the ventricles (*Masius, 1889; Barry, 1948*). Before the chick's seventy-second incubation hour, cells start to invade the cardiac jelly (*Baitsell, 1925; Garrault, 1934; Patten, Kramer, and Barry, 1948*) and gradually replace it with a cellular network. This process is illustrated in Fig. 285, which shows three stages at low and high magnification. In the opinion of *Chang (1932)*, the invading cells migrate inward from the epimyocardial layer; an endothelial origin was ascribed to them by *Patten, Kramer, and Barry (1948)*; and *Boerner-Patzelt (1931)* concluded, from studies of the



duck (*Anas platyrhynchos*) embryo, that they arise from both layers, but predominantly from the epimyocardium. In the regions of greatest endocardial thickening, cellular proliferation continues for a longer time than elsewhere (*Chang, 1932*). In the sinus venosus and the atria, the endothelium is closely applied to the myocardium (*Masius, 1889*). Within the ventricular cavities, the endothelium begins to be forced down between the projecting strands of cells (early trabeculae) at the end of the chick's third incubation day and is in close contact with the trabecular surfaces by the middle of the fifth day (*Kurkiewicz, 1910*). Endocardial connective tissue develops later.



**Fig. 285.** Changes in the histological structure of the cardiac jelly in the chick embryo's heart during the second, third, and fourth days of incubation. (Redrawn with modifications A, B, and C, after *Baitsell, 1925*; D, E, F, after *Patten, Kramer, and Barry, 1948*.)

A, at 27 hours ( $\times 125$ ); B, at 42 hours ( $\times 125$ ); C, at 72 hours ( $\times 175$ ); D, at 36 hours ( $\times 225$ ); E, at 65 hours ( $\times 225$ ); F, at 84 hours ( $\times 225$ ).

1, endocardium; 2, myocardium; 3, cardiac jelly; 4, cells invading the cardiac jelly.

### *The Myocardium*

The myocardium is composed of striated, seemingly syncytial muscle tissue supported by connective tissue containing a capillary network. The muscle fibers of the heart consist of cells on or near the surface of which there are parallel, longitudinally coursing myofibrils that apparently pass through several nuclear fields. Each myofibril exhibits transverse bands ("disks") that differ in refractivity and staining properties. An anisotropic, dark-staining *Q* band alternates with an isotropic, colorless *J* band of much the same width. The alignment of the *Q* and *J* bands with the corresponding bands in contiguous myofibrils imparts a cross-striated appearance to each



cell. Equidistant from either end of every *I* band is the dark, narrow *Z* band which traverses not only the myofibrils but also the sarcoplasm (cytoplasm) between them and thus unites the myofibrils. In all these structural details, cardiac muscle resembles skeletal muscle. A distinguishing feature of cardiac muscle is the presence of the so-called intercalated disks, which are short bands scattered at intervals. These are also transverse to the myofibrils, which they seem to gather together slightly.

The myocardium is the direct derivative of the thickened splanchnic mesoderm that gives rise to the outer mantle of the tubular heart. The thickness of the earliest cardiac primordia results from an increase in the height of a single layer of cells rather than from cellular proliferation. Two layers of cells, however, can be seen at the stage of 8 or 9 somites, three layers at that of 21 somites (*Bruno, 1918*), and four or five layers at the 3-day stage (*Schockaert, 1909*), after which the myocardium increases rapidly in thickness. During the third or fourth day, trabeculae start to project into the ventricular cavity, later to be largely replaced by compact muscular tissue. *Kurkiewicz (1910)* believed that the compact muscle is largely derived from the outermost flattened cells of the myocardium, visible at the 8-somite stage, that are usually considered to be early epicardial cells. According to his description, the single layer of superficial, flat elements separates from the underlying layers at the 2.5-day stage and immediately begins to proliferate cells into the intervening space, so that a reticulum is formed by the 3-day stage. He felt that this reticulum condensed to form compact muscle.

The structural characteristics of the myocardial cells undergo marked changes early in cardiac development. The earliest recognizable cardiac anlagen are composed of tall, loosely disposed, epithelioid elements whose long axes are perpendicular to the surface of the splanchnopleure. Between the 9- and the 17-somite stages (Fig. 286), the inner cells of the chick's two-layered epimyocardial mantle decrease in height and become round or elliptical (*Kurkiewicz, 1910; Bruno, 1918; Levi, 1922*). *Schockaert (1909)* stated that the myocardial cells in the 2-day chick embryo appear elongated and fusiform when sectioned longitudinally. The nucleus is oval (or round, in transverse section) and contains two or three nucleoli. Two diplosomes and numerous filaments are present in the granular cytoplasm. In tangential section, the cells appear to form a rather loose network in which there are many intercellular spaces (*Assaky, 1883; Schockaert, 1909; Congdon, 1918*).

Isolated myofibrils (Fig. 286-B) begin to appear at the 9- or 10-somite stage (*Kurkiewicz, 1910; Bruno, 1918; Lewis, 1919b; Levi, 1922; Olivo, 1925a*). Their number increases until, by the 21-somite stage (Fig. 286-D), they are quite abundant. Their differentiation continues throughout the greater part of the incubation period.



It has frequently been found that cell boundaries disappear during the period when myofibrils begin to differentiate. Many authors have noted that the cells of the epimyocardial mantle in the chick embryo are fused into a syncytium at a very early period (Rouget, 1863; Wagener, 1872; Schlater, 1906; Kurkiewicz, 1910; Duesberg, 1910; Congdon, 1918; Levi, 1919; Lewis, 1919b). Heidenhain (1899) pictured the myocardium of the 3-day duck (*Anas platyrhynchos*) embryo's heart as a syncytium traversed by long fibrils (Fig. 286-E). On the other hand, a few observers have denied that the embryo chick's heart muscle is truly syncytial (Wieman, 1907;

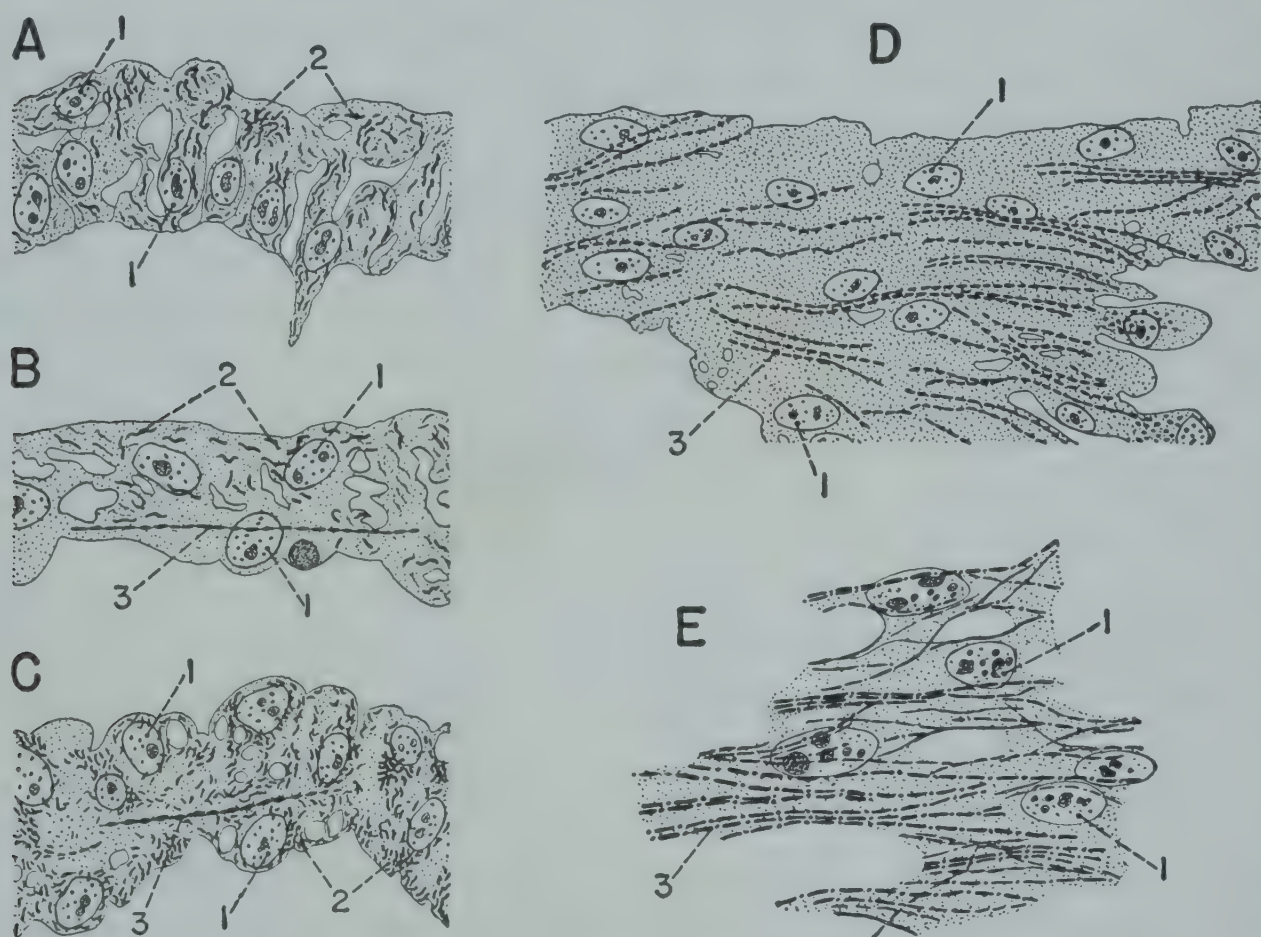


Fig. 286. Stages in the histological differentiation of myocardial tissue in the heart of the avian embryo. (Redrawn with modifications A, B, C, D, after Bruno, 1918; E, after Heidenhain, 1899.)

A, B, C, cross sections from the heart of the chick embryo of 9, 10, and 13 somites respectively; D, E, tangential sections from the 21-somite chick embryo's heart and the 3-day duck (*Anas platyrhynchos*) embryo's heart, respectively. All  $\times 1000$ .

1, nucleus of myocardial cell; 2, chondriomes (mitochondria); 3, myofibril.

Schockaert, 1909; Lewis, 1926). The appearance and the independent contraction rates of embryonic myocardial cells in culture led Lewis (1926) to the conclusion that the chick's cardiac muscle is a reticulum of anastomosed cells, rather than a syncytium. Schockaert (1909) stated that cellular individuality is masked at 4- to 6-day stages by the continuity of fibrils from cell to cell but becomes apparent during mitosis, when the cell membrane is clearly visible.

The origin and significance of myofibrils is not definitely known; in fact, their actual existence in the living cell has been questioned (Lewis, 1919a,



1919b; Lewis, 1926). It appears, however, that they are not produced by fixation, for they have been seen in living cardiac explants taken from 7- and 8-day chick embryos and grown *in vitro* (Olivo, 1923; Hogue, 1937), and also in the living heart of the 10- to 14-somite chick (Levi, 1922); they are revealed especially well by polarized light (Olivo, 1932). The effect of fixation is probably to bring the individual fibrils closer together (Renyi and Hogue, 1938).

It has been suggested that myofibrils represent only a highly specialized form of the contractile substance of the cell (Renyi and Hogue, 1938). The majority of investigators, however, have believed that myofibrils are derived from mitochondrial or other granules, or are formed under the influence of mitochondria. Schockaert (1909) claimed that small mitochondrial granules align themselves in rows and become transformed into homogeneous filaments which then elongate and finally differentiate as striated fibrils. Meves (1908) and Duesberg (1910) held the similar opinion that fibrils evolve from chondriosomes, filamentous bodies homologous to mitochondria. According to Bruno (1918), these filaments are abundant in the chick embryo's heart until the 15-somite stage but disappear by the 21-somite stage, when myofibrils become fairly numerous (cf. Fig. 286-D). The appearance of striated myofibrils coincident with the disappearance of mitochondrial filaments was also noted by Stilwell (1938). From the observation of heart muscle differentiation in culture, Stilwell (1938) concluded that mitochondria are present in the nondifferentiated myocardial cell in the form of both granules and short rods, and that it is the latter which give rise to the filaments, hence to the myofibrils. Furthermore, it has been found that the cells proliferated by cultured explants of the chick embryo's heart eventually dedifferentiate, or return to an indifferent state, and that during the course of dedifferentiation the myofibrils are replaced by long filaments which in turn give way to short rods (Levi, 1919, 1923; Olivo, 1923, 1925b, 1926c, 1929).

It has also been proposed that the axes of the myofibrils arise from a cytoplasmic reticulum (Wieman, 1907) or from a system of intersecting planes demarcating hexahedral compartments in the cytoplasm (Congdon, 1918). The cross striations are supposedly produced by the deposition of mitochondrial or other granules at the points of intersection of the meshes or planes.

Tension and mechanical stress have been thought to favor the formation of fibrils in living cultures of chick embryo heart muscle (Lewis, 1926; Stilwell, 1938) and hence, by inference, in this tissue *in vivo* also. However, when myocardial cells containing a few fibrils are stretched with microneedles for 5 to 8 minutes, additional fibrils do not form (Renyi and Hogue, 1938).

According to some investigators, the first fibrils to develop (seen in the



2.5- to 3-day chick embryo) are not striated, but homogeneous (Schlater, 1906; Schockaert, 1909; Duesberg, 1910). An ultrasensitive polarizing microscope has revealed birefringent smooth myofibrils at the 8- to 9-somite stage (Baud and Haenni, 1952).

It has also been said, however, that the earliest fibrils exhibit striations or are moniliform. Many authors have stated that the *Q* and *J* bands are the first to become visible, but the time of their appearance has been placed at various stages, including that of 36 to 41 hours, or 10 somites (Rouget, 1863; Kurkiewicz, 1910; Bruno, 1918); 4 to 6 days (Schockaert, 1909); 5.5 to 6 days (Wieman, 1907); and 7 days (Schlater, 1906). Stilwell (1938) noted the initial development of the *Z* band at the 8-day stage. Heidenhain (1899), however, found the *Z* band as well as the *Q* and *J* bands in the heart of the 3-day duck embryo, *Anas platyrhynchos* (cf. Fig. 286-E). The presence of these three bands in the myocardium of the 60-hour chick embryo was observed by Duesberg (1910), who remarked that the *Q* bands appear first as swellings spaced regularly along homogeneous fibrils, and that the *Z* bands develop almost simultaneously midway in the spaces between the *Q* bands. An even more precocious development of the *Z* band was reported by Lewis (1919a), whose examination of both fixed and living cardiac tissue from the chick embryo revealed that *Q*, *J*, and *Z* bands (but no fibrils) are apparently all present at the 10-somite stage. The polarizing microscope reveals the first indication of transverse striation at the stage of 12 somites (Baud and Haenni, 1952).

Little is known of the development of the intercalated disks. They appear in the chick embryo's heart late in incubation (Congdon, 1918), perhaps not until the last day (Hogue, 1947).

The development of myofibrils obviously does not depend upon contractile activity. Although myofibrils first become demonstrable at approximately the same time that contraction begins, they are present initially in very small number. Even at the 8-day stage, when heart function is well established, many nondifferentiated cells may still be seen in the myocardium (Stilwell, 1938). Furthermore, the contraction of explanted fragments of the heart is prevented by the presence of an excessive amount of potassium chloride in the culture medium, yet myofibrils continue to differentiate in the fragment (Olivo, 1928c).

The effect of certain cations on the morphology of myofibrils has been determined by culturing the chick embryo's heart in media containing the cations in excess. Potassium chloride has been found to cause no change in myofibrillar structure (Olivo, 1924a; Sacerdote de Lustig, 1942) but to produce a marked vacuolization of the cytoplasm (Olivo, 1924a; Bucciardi and Bisceglie, 1929); calcium chloride does not modify cellular morphology in any way (Bucciardi and Bisceglie, 1929). On the other hand, barium chloride either prevents myofibrils from differentiating in explants of young



hearts or destroys them in explants of older hearts (*Sacerdote de Lustig*, 1942).

*The dedifferentiation of cultured myocardium.* The dedifferentiation of embryonic heart muscle *in vitro*, previously mentioned, must be discussed briefly. The extent to which this process occurs is not definitely established. According to one viewpoint, the muscle cells of cultured embryonic heart tissue disappear completely after a few transplantations and are replaced by fibroblasts, which are cells derived from the mesenchymal connective tissue of the heart; thus Carrel and his associates usually referred to "strains of connective tissue cells" in reporting experiments performed over a period of many years on subcultures of a fragment taken in 1912 from the heart of the 7-day chick embryo. In the opinion of Levi (1923, 1934) and Olivo (1923, 1925*b*, 1926*c*, 1926*d*, 1929), the myocardial elements lose all their identifying characteristics after a few transplantations and can no longer be distinguished from fibroblasts. The loss of structural specificity is accompanied by a reduction in biological value, so that heart muscle cells eventually become indifferent both morphologically and functionally.

Dedifferentiation of heart muscle involves not only the loss of myofibrils but also the resolution of the myocardial syncytium. The process is completed first at the periphery of a culture. In describing the appearance of tissue from the 7-day chick embryo's heart grown for 2 days in chicken plasma and chick embryo extract, Olivo (1923) noted that the explanted fragment, which was pulsating actively, was surrounded by a thinner ring of tissue that was migrating peripherally. Close to the explant, the surrounding tissue consisted of a syncytium several cell layers deep and contained many striated or moniliform fibrils directed radially. As it proceeded centrifugally, the sheet of tissue became progressively thinner, and the fibrils were less numerous, shorter, and increasingly devoid of striations. Close to the margin of the culture, the tissue was no longer syncytial, the cells were flattened, fusiform, and without specific character, and most of the myofibrils were replaced by long chondriosomes. At the periphery, the greatly flattened and distinctly separated cells contained short chondrioconts and numerous fat droplets, but no myofibrils.

The time that elapses before all vestige of myofibrils disappears depends upon several factors, according to Olivo (1929). Dedifferentiation is more rapid in smaller pieces of tissue; but the most important determinants of its rate are the nature of the culture medium and the age of the embryo from which the explant is derived.

Dedifferentiation of myocardial tissue from chick embryos of all ages is accelerated by the presence of embryonic tissue extract, or "embryo juice," in the culture medium (Olivo, 1926*d*). Thus, in fragments of 6- to 8-day hearts grown in plasma and physiological salt solution, fibrils are present



for more than a week (Fig. 287-A), and some may persist for 2 weeks, after which the tissue degenerates; but when the culture medium consists of equal parts of plasma and embryonic extract (Fig. 287-B), a large proportion of the striated fibrils have disappeared before the first 24 hours *in vitro*, and practically none can be seen after 4 to 6 days (Olivo, 1926c).

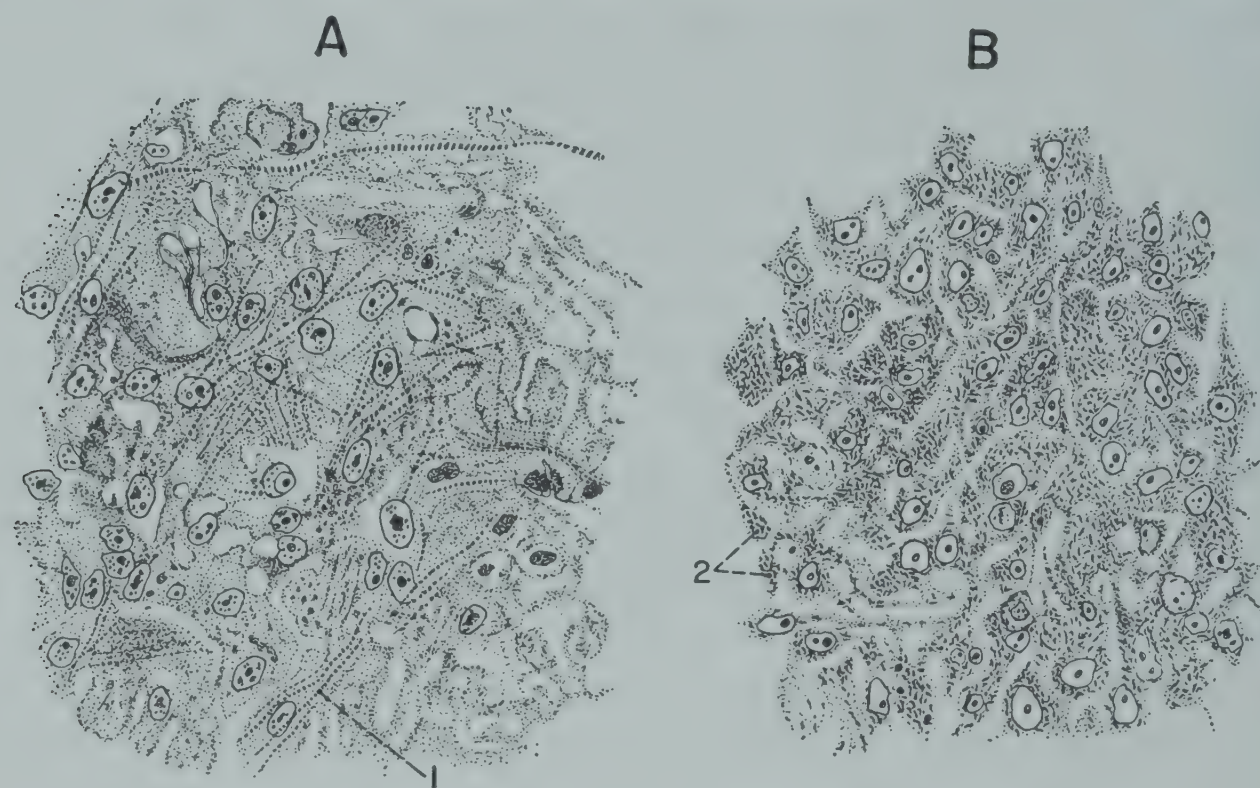


Fig. 287. Comparison of two sections of cultured myocardium from the 6-day chick embryo's heart, to show the accelerated dedifferentiation occurring in a medium containing embryonic tissue extract. (Redrawn with modifications after Olivo, 1926c.)

A, section of a fragment cultured in plasma and Ringer's solution for 11 days; B, section of a fragment from the same heart grown for the same period of time in equal parts of plasma and embryonic tissue extract. Both  $\times 500$ .

1, myofibril; 2, short chondriosomal filaments.

The time that myofibrils persist in cultures is inversely proportional to the age of the embryo from which the explant is removed. The tabulated data show the time required for dedifferentiation to occur in ventricular explants taken from embryos of various ages and grown in a medium consisting of plasma, Ringer's solution, and embryonic extract, in the ratio of 3:2:1, respectively (Olivo, 1929). Hogue (1937) claimed, however, that muscle cells from the 8-day chick embryo's heart remain identifiable after 6 months of culture in a medium consisting of Tyrode's solution, Locke-Lewis solution, and embryonic extract, in the ratio of 8:2:5, respectively.

Age of Embryo (days)	Persistence of Fibrils (days)
2-3	28
6	11
8	7
17	4



The relationship between embryonic age and the rate of dedifferentiation was explained by Olivo (1929) as being due not only to the greater ease with which younger tissue adapts itself to conditions *in vitro*, but also to the fact that the younger a ventricular fragment is at the time of explantation, the more undifferentiated cells it contains. Differentiation of these cells occurs after explantation, but at a slower rate than *in vivo*; hence dedifferentiation is also retarded. It follows, therefore, that the cells which eventually compose the outer portions of an embryonic heart culture consist of fibroblasts and dedifferentiated myoblasts in varying proportions, depending upon the incubation age of the original explant. In cultures of 6- to 10-day hearts, dedifferentiated myoblasts predominate, whereas fibroblasts do so in cultures from older hearts. This line of reasoning seems to be substantiated by an experiment performed by Törö (1941), who implanted fibroblasts from cultures of the chick embryo's heart into the eyes of living chickens, in the position normally occupied by the lens. Fibroblasts from 4-day hearts redifferentiated as myoblasts, whereas those from 6- to 14-day hearts gave rise to a mixture of myocardial and connective tissue cells, and those from still older hearts produced only connective tissue. The term "heart fibroblast," therefore, appears capable of being interpreted variously. It may be added that the redifferentiation of previously dedifferentiated myoblasts indicates that dedifferentiation *in vitro* may not necessarily imply a degradation of biological value, as suggested by Olivo (1929), but merely that the conditions of culture do not permit the different potencies of cells to remain in evidence.

It has been observed that fibroblasts of chick embryo heart origin may spontaneously transform themselves into macrophages after growth has ceased (Thomas, 1934b, 1937, 1938). Transformation may occur without cell multiplication but more often involves the emission of globular masses of protoplasm and the amitotic division of the nucleus. The addition of a drop of M/100 sodium hydroxide in Ringer's solution to a culture sometimes provokes the transformation (Thomas, 1936).

### *The Epicardium*

The epicardium, which is continuous with the pericardium, is structurally similar to the endocardium; it is an elastic connective tissue covered externally by a single layer of flat epithelial cells. It is generally stated in the literature that the epicardium differentiates at an early stage on the outer surface of the epimyocardium. Kurkiewicz (1910), however, claimed that the epithelial portion of the epicardium originates from the outer of two cell layers forming the wall of the sinus venosus. It was his opinion that the epicardial epithelium begins to be proliferated from the ventral surface of the sinus on the chick's third incubation day and gradually envelops the heart, and that the connective tissue elements invade the space



between the epicardial epithelium and the myocardium during the fifth day. He noted the presence of a layer of flat cells on the outer surface of the epimyocardial mantle of the 8-somite embryo's heart, but he regarded them as myocardial cells because he identified myofibrils in them in the middle of the third day. At this time, he found the two layers separated by a space, into which he observed cells from the outer layer migrating later in the same day. According to Bruno (1918), the differentiation of an outer layer of flattened cells, representing the epicardium, is barely beginning at the 13-somite stage but has reached completion by the 21-somite stage. This author remarked that the epicardium is everywhere closely adherent to the myocardium in the 3-day embryo.

The Growth of the Heart

Quantitative data on the growth of the chick embryo's heart reveal various interesting relationships. Some pertinent information is included in the accompanying table of the chick embryo compiled from the data of

Age of Embryo (days)	Dimensions of Heart	
	Length (mm.)	Width (mm.)
2	0.6	0.4
3	1.4	0.9
4-5	1.8	1.6
6-7	3.0	2.3
8-9	4.3	3.3
10-11	5.5	4.3
12-13	6.5	5.3
14-15	7.5	6.2
16-17	8.5	6.7
18-19	9.8	7.8
20-21	10.8	8.9

Romanoff (*unpublished*) except for the 2- and 3-day embryo (Moleschott, 1868). This table shows that the tubular heart more than doubles in length and breadth from the second to the third incubation day and that the chambered heart, from the fifth day to the end of incubation, becomes almost 6 times longer and about 4.5 times wider. Dimensional gains are of insignificant magnitude, however, when compared with the increase in weight, for the heart at the time of hatching is more than 2000 times heavier than on the third incubation day (see Appendix Table VI). The absolute weight of the heart increases gradually until the beginning of the third week, when the actual weight increments per day suddenly become larger; weight then increases linearly at a rate that continues unchanged at least until the eighteenth post-hatching day, except for a temporary disturbance immediately after hatching (Latimer, 1928). The daily percentage



increments in the weight of the heart are greatest, however, at the beginning of incubation; they decrease very rapidly during the first week and gradually but irregularly thereafter. In terms of percentage of total body weight, the weight of the heart increases only until the fourth day of development and then continually diminishes. Olivo (1933) detected sudden elevations in the weight of the heart relative to body weight not only on the fourth day but also on the eleventh and seventeenth days. He concluded that these three spurts in growth result from successive demands made on the heart, first by the development of the vitelline circulation, then by the development of the allantoic circulation, and finally by the initiation of pulmonary respiration.

It has been suggested that the chick's heart grows by cell multiplication until 1 or 2 days before hatching and increases in size thereafter principally by enlargement of the cells themselves (*Olivo and Slavich, 1930a*). This conclusion was reached through studies of mitotic activity in cardiac tissue of embryonic and young hatched chicks (*Olivo and Slavich, 1928, 1930a; Olivo, 1931a*). It was observed that the coefficient of mitosis, or mitotic index (the number of dividing cells per 100 resting cells) decreases rapidly and progressively from the second incubation day to hatching time and eventually becomes zero on the tenth day of postembryonic life; the values are given in the tabulation (*Olivo, 1928d; Olivo and Slavich, 1930a*).

Incubation Age (days)	Mitotic Index
2	2.25
3	2.12
5	1.69
7	1.51
9	0.83
11	1.00
13	0.70
15	0.44
17	0.37
19	0.28
21	0.12
10-day chick	0.00

The average duration of mitosis, calculated by formulas based on the mitotic index and the actual daily weight increases, was found to be 38 minutes. By the use of formulas involving the mitotic index, the weight increment, and the duration of mitosis, it was possible to calculate the average time required for the number of cells in a given field to double, which is essentially the average interval between two successive divisions of the same cell (assuming that all cells exhibit the same mitotic activity and that no cell divides a second time until all the others have divided). This intermitotic interval increases from about 20 hours on the second incuba-



tion day to 15 days at the time of hatching. Furthermore, the percentage increase in the weight of the heart for a unit of time equal to the duration of mitosis parallels the coefficient of mitosis only until the nineteenth incubation day and has a higher value thereafter. The terminal divergence was interpreted as indicating that some factor other than cell division accounts for cardiac growth at the end of the embryonic period and immediately afterward. The viewpoint that cellular hypertrophy is this factor receives support from the observation that the distribution of nuclei in cardiac tissue is much less dense 2 to 4 weeks after hatching than at the midpoint of incubation (*Olivo and Slavich, 1930a*).

The myoblasts of the embryonic chick heart exhibit a higher mitotic coefficient than the epithelial cells of the liver. Since the duration of mitosis is longer in the heart, the liver shows a higher hourly mitotic coefficient (the coefficient of mitosis divided by the duration of mitosis), especially during the first 12 days of the incubation period (*Olivo and Porta, 1931*). On the other hand, the velocity of the final phases of mitosis (*in vitro*) is considerably higher in chick heart myoblasts than in the endothelial cells of the liver (*Bucciante, 1943b*).

#### *The Influence of Temperature on Growth and Survival*

Incubation at subnormal temperatures disturbs the rate of increase in cardiac weight (*Olivo, 1931a*). Constant incubation temperatures of 36.5° C. and 34.5° C. result in a retardation in the growth of the heart for the greater part of the incubation period, which, at these temperatures, is extended by 4 and 5 days, respectively. However, on the sixteenth day at 36.5° C., or on the nineteenth day at 34.5° C., the heart makes a transition from a retarded to an accelerated growth rate, with the result that it is considerably heavier at hatching than it is when the embryo has been incubated at 39.5° C. (Fig. 288). The heart attains a greater final weight at 36.5° C. than at 34.5° C. (0.3760 gm., as compared with 0.2869 gm.). *Olivo (1931a)* explained the effect of lowered temperature on the growth of the heart in terms of increased frequency of cell division during the latter part of incubation, succeeding an earlier period of decreased frequency (cf. Fig. 288). This explanation was based on comparisons of mitotic coefficients.

The intrinsic viability of the chick embryo's heart, as distinct from the viability of the embryo as a whole, varies with temperature. The technique of tissue culture has made it possible to investigate the length of time that embryonic cardiac tissue, *in situ* or *in vitro*, can live at abnormal temperatures. Five amido-containing compounds were tested and compared with ethylene glycol and glycerol for protective effect on pieces of chick embryo heart, incubated 10 to 15 days, in culture against injury by freezing. Immersion in solutions of the compounds before freezing in liquid nitrogen



TABLE 12

## Viability of the 6- to 13-day Chick Embryo's Heart at Various Temperatures

Temperature (° C.)	Age of Embryo (days)	Survival Time		Investigator
		<i>In Situ</i>	<i>In Vitro</i>	
—195	7–15		1 min.¶	Luyet and Gonzales (1951)
—70	11	1 hr.†	1–2 hr.§	Hetherington and Craig (1939)
—60	7–12	1.5 hr.		Bucciante (1943a)
—25	6–12	0		Bucciante (1931)
—20	11	1.5 hr.†		Hetherington and Craig (1939)
—14	6–12	11 hr.		Bucciante (1931)
—10	6–12	16 hr.		Bucciante (1931)
—7	11	8 hr.†		Hetherington and Craig (1939)
—6– —7	?	8 hr.¶	8 days	Lambert (1913)
—4– —6	6–12	18 hr.		Bucciante (1931)
0– —1	?		10 days	Lambert (1913)
0	6–12	10 days		Bucciante (1931)
0	11	12–13 days †	15–16 days §	Hetherington and Craig (1939)
0	9–13	13 days	12–16 days ‡	Bucciante (1933)
5	8–12		13 days ‡	Bucciante and Levi (1931)
5	9–13	10–13 days	14–16 days ‡	Bucciante (1933)
6–7	?		18 days	Lambert (1913)
10	9–13	8–11 days	15–18 days ‡	Bucciante (1933)
12–14	?		12 days	Lambert (1913)
15–20	6–12	6 days		Bucciante (1931)
18–21	?		11 days	Lambert (1913)
20	9–13	6–7 days	10–12 days ‡	Bucciante (1933)
20	11	10–24 hr.†	6–11 days §	Hetherington and Craig (1939)
37.5–39	11	2.5–4 hr.†	3–8 days §	Hetherington and Craig (1939)
38	9	3.5–4 hr.*		Wilburg (1937)
38	9–13		4–6 days ‡	Bucciante (1933)
45.5	20	1–3 hr.		Szarski (1948)
48	11	1.5 hr.†	5–20 hr.§	Hetherington and Craig (1939)
52	11	0.5 hr.†	2–2.5 hr.§	Hetherington and Craig (1939)

\* In exsanguinated embryo; † in embryo removed from egg; ‡ in Ringer's solution; § in Ringer-Tyrode solution; || in plasma, serum, Ringer's solution, or normal saline solution; ¶ after partial dehydration in ethylene glycol.



showed acetamide and urea had high efficiency over a limited range of concentration, 5.4 M and 2.5 M, respectively, being the optimum concentrations. Formamide, propionamide, and methylurea are relatively poor protective agents. Ethylene glycol and glycerol were equally effective at all high concentrations (Keane, 1953). The test for survival is the ability of heart explants to grow at 37.5° C. after exposure of the heart to the desired temperature. Some of the results are summarized in Table 12. In gen-

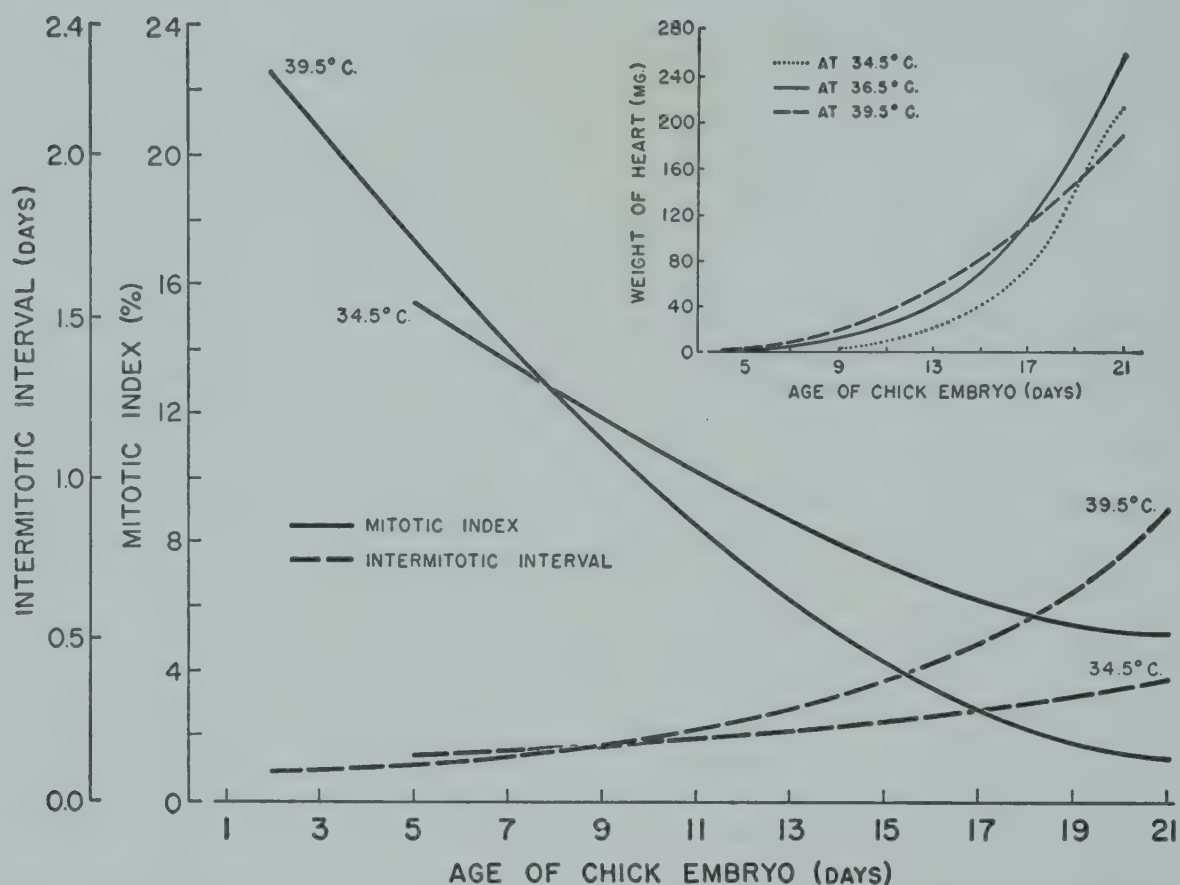


Fig. 288. The effect of incubation temperature on the mitotic index and intermitotic interval in cardiac tissue at various periods during the incubation of the chick embryo. Effect of temperature on the weight of the heart is shown in the *Insert*. (Redrawn with modifications after Olivo, 1931a.)

eral, excised hearts seem to live longer at abnormal temperatures than hearts left in place in the embryo; also, the survival time of an excised fragment is said to be inversely proportional to its size (Hetherington and Craig, 1939). The excised heart survives best at temperatures close to 10° C.; but when the embryo is left undisturbed in the egg, cardiac viability is greatest at 0° to 5° C. The ventricle has less resistance to temperatures between 0° C. and 38° C. than the atrium, both *in situ* and *in vitro* (Bucciante, 1933).

#### *The Growth of Embryonic Heart Tissue in Vitro*

Numerous investigators have described the growth of fragments of the chick embryo's heart *in vitro* as beginning a few hours after explantation with the centrifugal migration of fibroblasts. On or after the second day of culture, these are followed by migrating muscle cells. The explant soon becomes surrounded by a thin ring of cells which constitute the zone of



migration. Eventually, the muscle cells either dedifferentiate or give way entirely to fibroblasts.

The growth rate of heart tissue *in vitro* has often been measured by the increase in the area of the culture or in the width of the zone of migration. Growth may also be evaluated by the rate of cell division, expressed in terms of the mitotic index or coefficient. Neither of these criteria is entirely satisfactory, for it has been shown that cell migration can occur (and the culture therefore can appear to grow) either with or without a simultaneous increase in the number of cells (*Cunningham and Kirk, 1942*).

The results of numerous studies indicate that growth in cultures of the chick embryo's heart is influenced by many factors. Most of these are associated with conditions of the environment, including the chemical environment provided by the culture medium.

**The Intrinsic Characteristics of Growth *in Vitro*.** When tissue from the embryonic chick's heart is cultivated in the most favorable culture medium (a mixture of chicken blood plasma and chick embryo extract), growth follows a typical pattern.

Observations of mitotic activity in cardiac explants from embryos 2 to 17 days old indicate that cell division is very infrequent during the first 1 or 2 hours of culture. The mitotic index increases to a maximum between the third and fifth hours and subsequently diminishes to a very low value by the end of the twelfth hour. Neither the time at which migrating cells appear nor the widening of the zone of migration seems to be correlated with mitotic activity during the first 12 hours (*Olivo and Delorenzi, 1931*).

Within the first 48 hours, the mitotic index and the size of individual cells is distinctly greater near the periphery of the zone of migration than near the edge of the explant, probably because the medium is depleted of nutrients first in the regions where it has had longest contact with cells. These relationships no longer exist in 96-hour (untransplanted) cultures, either because the nutrients are approaching exhaustion or because the stimulating properties of the medium have decreased. The greatest number of cells per unit volume of tissue is found at the periphery of 24-hour cultures and midway between periphery and explant in 48-, 72-, and 96-hour (untransplanted) cultures; were it not for depletion of nutrients toward the center, cell density would be greatest in the oldest (central) part of the zone of migration (*Tompkins, Cunningham, and Kirk, 1947*).

The time that elapses before migration begins (the latent period) is directly related to the age of the embryo when the heart fragment is removed. In cultures of 3- to 5-day hearts, cells leave the explant within the first 3 hours; in cultures of 9- to 11-day hearts, they do so within 7 hours; and in cultures of 18-day hearts, they do not migrate out until the tenth hour. During the first 24 hours *in vitro*, therefore, there is an inverse relationship between embryonic age and the increase in the surface area of



the culture (*Olivo and Delorenzi, 1931*). Nemoto (1929) observed that the latent period decreases by 1 or 2 hours with every successive transplantation of a culture to fresh medium.

Comparison of the coefficients of mitosis of embryonic chick heart tissue *in vivo* and *in vitro* (*Olivo, 1928d; Olivo and Slavich, 1928, 1930b*) has shown that mitotic activity is much lower in an explant than in a normally developing heart of the same age, whether this be 3, 7, or 15 days. The younger the embryo from which the tissue is taken—that is, the higher the mitotic activity at the time of removal—the greater is the relative decrease in mitotic activity, as shown by the average mitotic coefficient for the first 48 hours after explantation. The accompanying data (*Olivo, 1928d*) indicate this relationship.

Age of Embryo (days)	Mitotic Coefficient	
	<i>In vivo</i>	<i>In vitro</i>
3	2.12	0.62
7	1.51	0.76
15	0.44	0.30

Transplanting the tissue to fresh medium at the end of 48 hours causes a temporary rise in mitotic activity followed by a progressive decrease, so that the average mitotic coefficient is lower. This is true, however, only for the first two transplants. With every subsequent transplantation the average mitotic coefficient rises, so that eventually it not only surpasses its value for the first 48 hours but also attains a value equal to that of heart tissue *in vivo* at a corresponding age—that is, 6 or 8 days older than the age at explantation (*Olivo and Slavich, 1930b*). This increase in mitotic activity is supposedly correlated with the onset of dedifferentiation. In the zone of migration, composed largely of fibroblasts or dedifferentiated cells, the mitotic index is high, varying between 2.1 and 3.2 in successive passages of 7-day heart tissue; but the index is only 0.76 in the explant itself (*Olivo, 1928d; Olivo and Delorenzi, 1932*). The intermitotic interval for the migrating cells ranges from 12 to 18 hours. It is apparent, therefore, that the migrating cells in cultures of the 7-day heart are even more active mitotically than the cells of the 2-day heart *in vivo*. This is consistent with the observation that the growth rate of heart fibroblasts, cultured for months or years in the proper medium, is so high that the mass of tissue is doubled every 48 hours (*Carrel, 1914; Ebeling, 1922*).

**External Physical Factors Influencing Growth and Survival *in Vitro*.** It is to be expected that various aspects of the external physical environment would be found to modify the growth of cultured embryonic heart tissue. Various forms of energy, such as heat, light, and X-rays have their effect.

The minimum survival time of chick heart fibroblasts at various tem-



peratures is shown in the tabulation. The data for temperatures below 0° C. are those of Lambert and Hanes (1913); the remaining data are those of Nemoto (1929), except the value for 50° C., which was observed by Kemp and Juul (1931). In addition, Szarski (1946) found that cells in the zone of migration of (11-day) heart cultures are all killed by a temperature of 48° C. applied for 36 to 50 minues, but that the cells in the center of the culture seem to be more resistant, in view of the fact that migrating cells later appear in cultures held at this temperature for 1 to 2 hours.

Temperature (°C.)	Survival Time (hours)
—20	0.08–0.17
—10	2
—4	24–96
5	72
12	144
20	264
30	288
38–39	168
45	24
45–47	0.75
50	0.34

The lower and upper limits of continuously applied temperature permitting the growth of chick heart “connective tissue” have been placed at 26° C. and 44° C. or 45° C., respectively (*Lambert and Hanes, 1913; Nemoto, 1929*). Fischer (1926) observed, however, that both fibroblasts and freshly explanted heart tissue grew in plasma and embryo juice during a month’s sojourn at 20° C. in the dark. Growth is maximal at 36° to 39° C. (*Lambert and Hanes, 1913; Nemoto, 1929*) and falls off as the temperature deviates in either direction. More growth (of fibroblasts) has been said to take place at 30° C. than at 45° C. (*Nemoto, 1929*). The time required for cell division is twice as long at 28° C. as at 38° C. (*Lambert and Hanes, 1913*).

The influence of 5-minute exposures to temperatures of 45° to 50° C., inclusive, on the processes of growth and cell division in heart fibroblast cultures was investigated by Kemp and Juul (1931). At 50° C., probably only the dividing cells are killed in 5 minutes. After heating at 48° C., cell division is almost entirely suppressed for 3 hours, but the number of mitoses increases to about 160 per cent of normal by the end of the next 3 hours. One hour after heating at 47° C., mitotic activity is only 50 per cent of normal, but this decrease is followed by recovery 5 hours later. A similar exposure to a temperature of 46° C. has no significant effect on the number of dividing cells. Study of tissues fixed 12 to 60 minutes after the application of heat for 5 minutes indicated that, at 48° C., prophase is inhibited



and the completion of anaphase prevented (Fig. 289). At 47° C., anaphase can be completed, but the transition from metaphase to anaphase is blocked. At 46° C., prophase is no longer impossible; and at 45° C., the cells are once more able to pass from metaphase to anaphase.

Incubation of chick embryo heart cultures at 42° C. in a medium of plasma and embryo juice is conducive to the production of multipolar mitoses (*Stilwell, 1947*). Most of the abnormal mitoses appear to occur in fibroblasts rather than in myoblasts (*Stilwell, 1944*). A long exposure (several days) to a temperature of 42° C. produces more multipolar mitoses than exposures of a half hour to 3 hours to the higher temperatures of

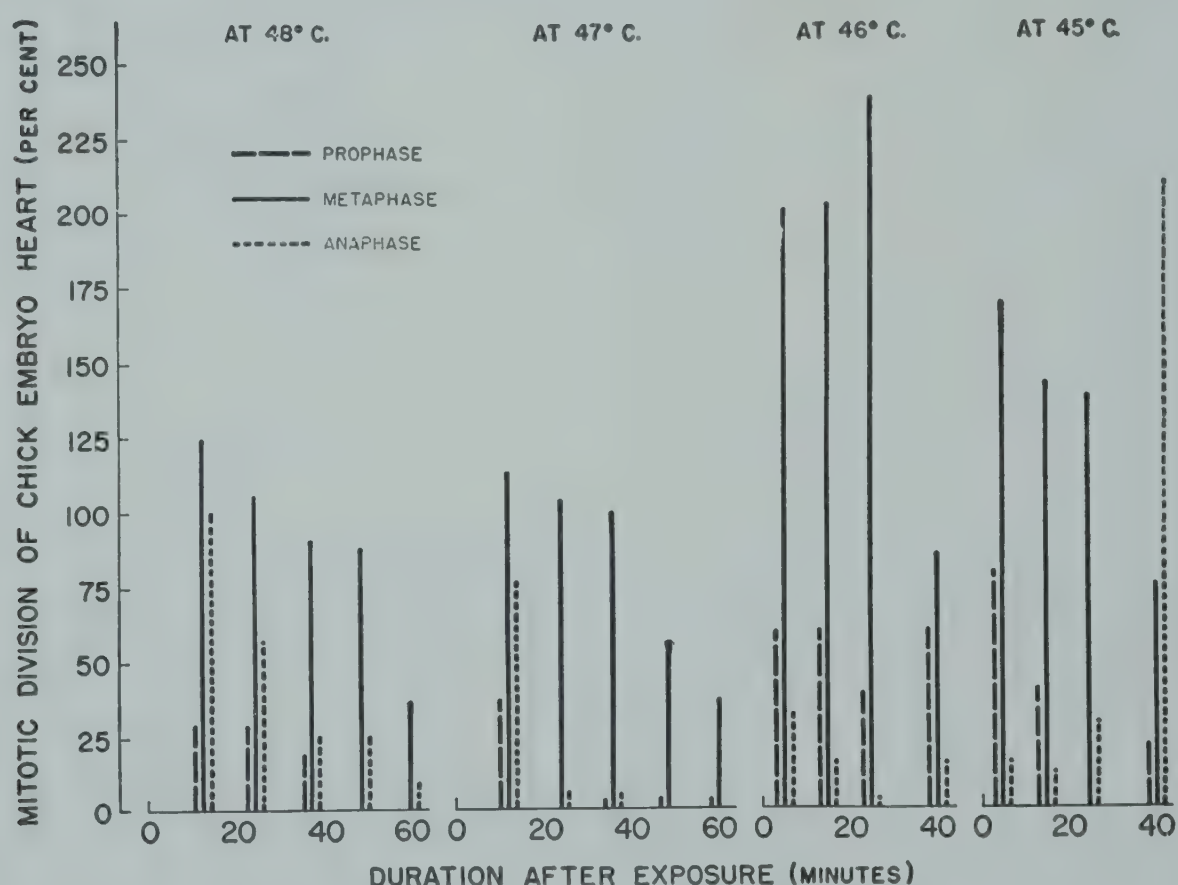


Fig. 289. The effect of 5-minute periods of overheating at four different temperatures on prophase, metaphase, and anaphase of mitotic division, as shown by observations during the first hour after exposure to heat. (Redrawn with modifications after Kemp and Juul, 1931.)

44° C. and 46° C. (*Stilwell, 1952a*). In heart cultures grown at 42° to 43° C. for 8 days, there may be a few cells in the marginal zone in which there is endomitosis, or reduplication and division of the chromosomes within an intact nuclear membrane (*Stilwell, 1952b*).

X-ray irradiation retards the growth rate in cultures of the embryonic chick heart (*Wendrowsky and Zapolska, 1935*) and causes a decrease in mitotic activity in fibroblast cultures. Kemp (1931) administered 150 r for 3 minutes to cultures derived from the 10- to 12-day chick heart and was unable to find any mitotic figures 4 hours later. At the end of 6 hours, mitotic activity was 59 per cent of normal, and at the end of 20 hours it was only 20 per cent of normal. In tissues fixed at several intervals within



the first 48 minutes after treatment with 1000 *r*, cells in prophase were the first to disappear, then those in metaphase, and lastly those in anaphase (*Kemp and Juul, 1931*).

Irradiation of the 6- or 7-day embryo (with 2000 *r* of X-rays) before explantation of myocardial fragments results in a prolonged latent period prior to the migration of cells in cultures of heart tissue. It appears that some cells are irradiated at a susceptible phase and die. After 12 hours of culture, however, the initial delay is made up, since the unaffected cells develop compensatory growth (*Rondinini and Monetti, 1952*).

Exposure to weak radium emanations for 10 to 15 minutes transforms the chondrioconts of dedifferentiated embryonic heart cells into mitochondria. Longer exposure (40 minutes) also causes vacuoles to appear in the cytoplasm (*Bisceglie and Bucciardi, 1929*).

Pires Soares (1934) observed that heart cultures from the 6-day embryo have an increased survival time when grown in the light of a 40-watt electric bulb placed behind a filter of copper sulfate solution.

**The Influence of the Culture Medium.** The chemical environment in which a tissue explant is cultivated is of utmost importance. The chemical environment, of course, is the culture medium. Assuming that a proper substrate is provided, along with water and a means of eliminating the products of metabolism, the essential requirement of explanted cells is utilizable nutrient material presented under the proper conditions of osmotic tension and hydrogen-ion concentration.

**Hydrogen-ion concentration.** Fischer (1921) showed that the growth of chick heart fibroblasts is markedly modified by the hydrogen-ion concentration of the culture medium. His cultures were derived both from freshly extirpated hearts and from strains of cells cultivated *in vitro* for as long as 9 years. In these cultures, the area of the migration zone was always maximum when the *pH* of the medium (plasma and embryo juice) was between 7.4 and 7.8 and fell off sharply with any decrease or increase in *pH*. Growth was usually inhibited to a greater extent at *pH* 8.0 than at *pH* 6.0; yet the cultures could not survive more than four to six transplantations at *pH* 5.5, although they lived through eight to ten passages at *pH* 8.5. Paff (1946), who altered the *pH* of the media in roller tubes by modifying the carbon dioxide content of the air, obtained essentially similar results.

**Osmotic tension.** An increase or a decrease in the osmotic tension of the medium causes a temporary rise in the growth rate of chick heart fibroblasts, followed by a diminution to a level below normal in the first or second transplant. This effect is shown by measurements of the spread of cultures in hypertonic and hypotonic media composed of plasma and embryo juice (*Ebeling, 1914*) as well as by the mitotic index of 3-day embryo heart cultures in hypotonic plasma (*Olivo and Gomirato, 1932*).



Fibroblasts survive only a limited number of transplantations (twelve) in a hypertonic medium. The zone of migration is denser than normal when osmotic tension is increased, thinner than normal when it is decreased. Fat globules appear in the cells in both hypertonic and hypotonic media (Ebeling, 1914).

Hogue (1919), who studied the effects of hypertonic and hypotonic Locke-Lewis solutions, observed no initial acceleration of growth under increased osmotic tension. In hypertonic media the cells at the outer edge of the culture die first, whereas those nearest the explant are the first to die in hypotonic media.

Dilution of the medium—whether plasma (Levi, 1934), or plasma and embryo juice (Ebeling, 1914)—without changing the osmotic tension favors the migration of cells in all passages but causes no increase in the actual rate of cellular proliferation. However, in a medium consisting of only 5 per cent plasma in Ringer's solution, heart tissue from the 3-day or 7-day chick embryo shows a great reduction in mitotic activity, which ceases entirely after two transplantations. It was suggested that this effect is due not so much to the lack of nutritive substances as to the fact that the medium becomes strongly acid within 24 hours (Olivo and Gomirato, 1931).

**The nutrient substances of the medium.** As pointed out by Carrel and Ebeling (1923d), a distinction must be made between growth and survival *in vitro*. Heart tissue from chick embryos incubated less than 2 weeks has been found to grow fairly well for a few days in various nonnutritive salt solutions (Lewis and Lewis, 1911; Olivo, 1922; Heaton, 1926), but its ability to do so is probably due to the utilization of nutrients stored in the cells, or to the presence within them of growth-promoting substances common to all embryonic tissues. In chick heart fibroblast cultures grown in Tyrode's solution on a plasma clot, the mitotic index reaches zero after 40 to 70 hours (Willmer and Jacoby, 1936).

The usual culture media are plasma, serum, or embryonic tissue extract ("embryo juice"), mixed with each other or with various physiological salt solutions. These three media are all the products of cells; their exact chemical composition is unknown, hence the nature of the nutrients they supply is obscure. In fact, Carrel and Ebeling (1921a, 1923b, 1923d) concluded that serum and plasma provide no utilizable nutrients but, like salt solutions, merely permit survival; in their opinion, embryonic tissue extract alone contains true growth-promoting substances. An actual decrease in protein nitrogen has been found in chick heart fragments grown in serum and Tyrode's solution (Boyer and Kirk, 1952). There is some evidence, however, that plasma, serum, and embryo juice contain dialyzable accessory growth factors which activate the growth-promoting substances of embryo juice (Fischer, 1941a). Pires Soares (1947) proposed that plasma



lacks sulfhydryl groups but catalyzes the use of the abundant sulfhydryl groups contained in embryonic extract.

Plasma without embryo juice permits the growth of embryonic heart tissue for a limited time. In adult chicken plasma, heart cultures (from 10- to 14-day embryos) grow to a maximum size after four to six transplantations, then become smaller; finally, after ten to thirty passages, they die (*Carrel and Ebeling, 1921a*).

Clotted plasma has had very extensive use as a supporting framework for cultured tissues. Plasma clots deteriorate with age, and even when metabolic products are washed away, cultures must be transplanted. Olivo (*1931b*) has presented evidence that inhibition of growth can be caused by chemical changes within the plasma clot itself. Growth is retarded in cultures on plasma clots 1 to 4 months old even in the presence of fresh plasma and embryo juice; also, aqueous extracts of old plasma clots decrease growth and reduce the mitotic coefficient in fresh cultures.

Plasma varies in effectiveness as a culture medium according to the age of the animal from which it is derived. Carrel and Ebeling (*1921b*) showed that the width of the zone of migration in cultures of the chick embryo's heart is greater in plasma from chickens 4 months old than in plasma from chickens aged 2 and 5 years. This finding suggested to Andai (*1932*) the possibility that embryonic plasma might contain growth-promoting substances resembling those in embryo juice. Experiments with the plasma of 10- to 17-day chick embryos showed, however, that heart fibroblasts (obtained from 6-week-old cultures of the 12-day embryo's heart) cannot live for an indefinite number of passages in embryonic plasma as they can in media containing embryo juice. In general, cultures grow to a larger size and survive a considerably greater number of transplantations in embryonic plasma than in the plasma of chickens 10 months old. This limited growth-promoting capacity of embryonic plasma increases regularly from the tenth to the seventeenth day of incubation. The exact age when activity is maximum has not been determined.

According to Carrel and Ebeling (*1921b, 1923a, 1923b, 1923c*), the reduced growth of heart fibroblasts in plasma from older chickens is related to the presence of growth-inhibiting substances in the serum, or to an antagonism between growth-stimulating and growth-inhibiting substances. Twenty-five to fifty per cent serum added to Tyrode's solution or to embryo juice shortens the life span of fibroblasts. The substances precipitated by carbon dioxide from the serums of chickens 10 months to 2 years old have a slight growth-promoting effect on heart fibroblasts, but precipitates from the serums of 6-year-old birds are practically inactive. Unless derived from old chickens, serums from which such precipitates are removed show increased inhibitory activity. Heating serum at temperatures of 56° C. to 70° C. augments its inhibitory effect (*Carrel and Ebeling, 1922b*), to a



greater extent if it is the serum of a young chicken; but heating at 100° C. restores activity to the level of non-heated serum. Heart fibroblasts also grow less well in shaken chicken serum than in normal serum. From all the above evidence, it appears that serum contains more than one substance affecting growth. It is possible that the disappearance of growth-promoting principles and the enhanced activity of growth-inhibiting principles accounts for the increasingly inhibitory effect of serum from older chickens. Ether-soluble lipoids may be among the inhibitory substances, since the area of growth of heart fibroblasts in an emulsion of these lipoids is reduced to 70 per cent of the area observed in Tyrode's solution (*Baker and Carrel, 1925*).

Tissue derived from the chick embryo's heart can grow in plasma or serum from animals other than the chicken. It has been reported that cat or dog serum does not affect the growth of heart fibroblasts when present in small amounts (*Carrel and Ebeling, 1922a; Fischer, 1922*) but retards it in concentrations exceeding 15 to 25 per cent; 30 to 45 per cent dog serum or 55 to 60 per cent cat serum was found to be completely inhibitory (*Carrel and Ebeling, 1922a*). On the other hand, Iwanitzkaia (1939-40) observed that fragments of the 8- to 9-day chick embryo's heart can grow in undiluted cat, dog, or rabbit plasma, although not so well as in chicken plasma. As judged by the increase in the area of the cultures, as well as by several other criteria (see the data in the tabulation), growth is considerably better in the plasma of the rabbit than in that of the two other animals and is poorest in cat plasma. The morphology of the cells in the zone of migration is altered in heterologous plasmas, although less in rabbit than in cat and dog plasma. The addition of chick embryo extract to cat and dog plasma largely overcomes their growth-inhibiting effect.

Plasma	Latent Period (hours)	Non-Growing Cultures (per cent)	Mitotic Index (per cent)	Abnormal Mitoses (per cent)
Chicken	3	6.7	2.1	4.2
Rabbit	6	7.4	2.2	8.0
Dog	12	34.0	1.2	35.9
Cat	12	57.5	1.2	

The age of the animal from which heterologous serum is derived influences the growth of chick heart fibroblasts in the same manner as the age of the bird used as the source of chicken serum or plasma (*Carrel and Ebeling, 1922a*). However, heating dog, cat, or rabbit serum at 56° C. or 66° C. has results that are the reverse of those obtained with chicken serum; that is, the inhibiting effect of heterologous serum is reduced, rather than increased (*Carrel and Ebeling, 1922b, 1922c*), probably due to the destruc-



tion of alexin (*Carrel and Ebeling, 1922c*). Shaking similarly improves a foreign serum as a culture medium (*Carrel and Ebeling, 1922c*).

If chick heart fibroblasts are cultured for a time in a medium containing an amount (7 per cent) of foreign protein (such as dog serum or human ascitic fluid) that is too small to affect their growth, they can subsequently grow at a normal rate in the presence of as much as 50 per cent of the same foreign protein. It appears, therefore, that chick fibroblasts *in vitro* can become immunized to an antigen in the medium (*Fischer, 1922*).

The effectiveness of embryo extract in encouraging the growth of fibroblasts derived from the chick embryo's heart was first noted by Carrel (1913a). In fact, it appears that fibroblast cultures can live and grow indefinitely in the presence of embryonic tissue juice (*Carrel, 1913b; Ebeling, 1919, 1922*), provided they are frequently washed with Ringer's solution and transferred to fresh media. According to Stewart and Kirk (1952) growth indexes calculated from data showing the effect of eight different liquid media on 11.5-day chick embryo heart explants grown *in vitro* support the belief that a tissue extract must be present in the liquid medium if true tissue growth is to occur.

Embryonic tissue juice stimulates both the migration and the division of cells cultivated *in vitro*. Its effect on migration is shown by the observation that its presence in the medium leads to an increased rate of migration in cultures of heart fibroblasts irradiated with more than twice the dosage of X-rays required to prevent mitosis (*Doljanski and Goldhaber, 1945*). Carrel and Ebeling (1921a) found that the width of the zone of migration in heart fibroblast cultures is proportional to the content of embryo juice in the medium (serum), but that the relationship is not linear. The first 10 per cent of juice added is relatively more stimulating than the next 30 per cent, and a concentration of 40 per cent is as effective as all higher concentrations. Willmer and Jacoby (1936), who added embryo juice to heart cultures that had stopped growing in plasma clots in Tyrode's solution, reported that migration starts only 2 hours after the addition of embryo juice and increases in rate for several hours, the rate being proportional to the concentration of embryo juice. Mitoses were observed to recommence in these cultures 10 to 12 hours after the embryo juice was added and to increase in number for another 12 hours, after which time their frequency decreased. Under the conditions of this experiment, there was no particular correlation between the mitotic index and the concentration of embryo juice. Wright (1925), however, observed that the number of dividing fibroblasts in cultures of 9- to 12-day chick hearts is greatest in pure embryo juice and becomes progressively smaller upon dilution of embryo juice with one part and two parts of a saline solution. The favorable effect of the admixture of embryo juice with plasma in the ratio of 1:1



is illustrated by the data in the tabulation (*Olivo and Gomirato, 1931*), which compares the mitotic coefficients of five successive transplants of 7-day heart fragments in the two media.

Transplant Number	Mitotic Coefficient	
	In Plasma	In Plasma Plus Embryo Juice
1	0.53	0.76
2	0.23	0.66
3	0.04	0.41
4	0.04	0.45
5	0.05	0.50

Investigations by Kaufman (1926, 1927) indicate that duck (*Anas platyrhynchos*) embryo juice is intrinsically superior to chick embryo juice in promoting the growth (shown by size increase) of heart cultures from chick and duck embryos of 8 and 11 days' incubation, respectively. Chick heart cells become slightly larger than normal when grown in the presence of duck embryo juice.

All of the substances in embryonic tissue juice which are responsible for its favorable effect on cultures of heart (and other) tissue have not been identified at the present writing. It has been suggested that the growth-promoting principles are contained in the protein fraction (*Baker and Carrel, 1926*) and that they are proteoses, metaproteins (*Carrel and Baker, 1926a, 1926b*) or nucleoproteins (*Fischer, 1941b; Fischer and Astrup, 1943*). The growth-stimulating substances, which are heat-labile and of high molecular weight (*Carrel, 1913a*), have been grouped together under the name "embryonin" (*Fischer and Astrup, 1943*). It appears, however, that embryo juice and perhaps plasma and serum, also, contain certain additional substances of low molecular weight which can be removed by dialysis against a Ringer-glucose solution (*Fischer, 1941a*) and which not only maintain the cells in good condition but also activate embryonin. The nature of these substances is uncertain. According to Harris (1952), they are neither proteins nor lipids, they are partially heat-stable, and they are largely destroyed by acid hydrolysis. Attempts to identify them have involved investigation of the nutrient requirements of myoblasts and fibroblasts from the embryonic chick heart, but very few results have remained unchallenged. It has been proposed that the basal foods needed by these cells are organic phosphates, sugar, peptides, and amino acids (*Ehrensward, Fischer, and Stjernholm, 1949*), especially cystine, glycine, and glutamine (*Fischer, 1948*). Water-soluble vitamins do not appear to be growth stimulants (*Hengstman, 1938-39*). Among carbohydrates, glucose, fructose, and mannose are utilized (*Fischer, 1948; Spratt, 1950b; Harris and Kutsky, 1953*), but not lactose, saccharose, galactose, arabinose, sylose, L-glucose,



starch, glycogen, d-lactic acid, or pyruvic acid (*Fischer, 1948; Harris and Kutsky, 1953*); growth is retarded by glucosamine (*Ely, Tull, and Schanen, 1953*) and 2-desoxy-D-glucose (*Ely, Tull, and Hard, 1952*).

*The effect of miscellaneous substances.* Various substances of a non-nutritive nature have been observed to modify the growth of embryonic chick heart cultures.

Sodium para-aminosalicylate has been found to stimulate the growth of dedifferentiated 8-day chick heart myoblasts (as judged by increase in the size of the cultures) in all concentrations between 5 and 100 mg. per 100 cc. of culture medium, but it has a definitely inhibitory effect in concentrations of 300 mg. to 1000 mg. per 100 cc. (*Attardi, Viterbo, and Gandini, 1952*). Similarly, sodium pteroyltriglutamate is stimulating in concentrations of 0.01 to 1.0 micrograms per cc. and inhibitory in concentrations of 500 micrograms or more per cc. (*Attardi, 1951*).

Sulfanilamide (0.1 and 0.2 per cent) as 20 per cent of the medium produces intense cellular proliferation, particularly of fibroblasts; but after the third day, many cells in the zone of migration become transformed into macrophages (*Pires Soares, 1944b*). Streptomycin increases the number of pathological chromosomes when present in a concentration of 2500 micrograms per milliliter of culture medium (*Keilova-Rodova, 1951*). It markedly reduces growth in concentrations of 6000 to 15,000 micrograms per milliliter and is completely inhibitory at a concentration of 90,000 micrograms per milliliter. Chloromycetin disturbs mitosis (*Keilova-Rodova, 1951*) and retards growth in concentrations of 480 to 1200 micrograms per milliliter of medium (*Fusillo, Metzger, and Kuhns, 1952*). Aureomycin has similar effects, but patulin is much more active (*Keilova-Rodova, 1951*). A study by Zulj-Milković (1954) of the influence of streptomycin on the growth in tissue culture of fibroblasts of ninety 11-day chick embryo hearts showed marked stimulation of growth of fibroblasts.

In the clinical dosage, neither privine hydrochloride nor neosynephrine hydrochloride affects the growth of heart fibroblasts. Neosynephrine, though indifferent at a concentration of 1:2000, is inhibitory at one of 1:1000. Privine is harmless at a concentration of 1:10,000 but damages fibroblasts at a concentration of 1:5000 and completely prevents their growth at concentrations of 1:4000 or less (*Pomerat, Lay, and Emerson, 1944*).

The increase in the size of chick heart fibroblast cultures is not affected within 48 hours by the addition of 0.01 per cent phenergan but is limited to 12 to 51 per cent of normal in the presence of 0.25 per cent phenergan and to only 0.7 to 5 per cent of normal when the concentration of the drug is 0.031 to 0.037 per cent. Growth is totally inhibited by concentrations of more than 0.05 per cent (*Driessens, 1952*).

Treatment with iodine vapor may cause staining and some destruction and degeneration in cultures of heart fibroblasts. After several passages



through fresh media, however, cellular proliferation resumes (*Strickler and Fowler, 1933*).

Quinine in very dilute solution (1:10,000) considerably hinders the growth of heart fibroblasts; stronger solutions (1:5000) render growth impossible (*Partachnikov, 1930*). The effect of quinine on the growth of ventricular tissue from the chick embryo's heart was also noted by *Kamon (1928)*, who found the following compounds, in addition, to be inhibitory in appropriate quantities: digitalin, saponin, morphine, cocaine, atropine, pilocarpine, potassium cyanide, sodium arsenite, and ethyl alcohol.

The influence of various doses of seven narcotics on chick heart cultures was studied by *Takeguchi (1938)*. At a concentration of 0.05 per cent in a nutrient medium, avertin, tropococaine, and sodium evipan markedly inhibited growth, and pantocaine, nupercaine, scurocaine, and bancaine prevented it entirely. The adverse effect of all seven drugs was detectable though slight at concentrations of 0.00005 per cent.

Treatment with 0.1 or 1.0 per cent thiourea solution for no longer than 30 minutes has a somewhat retarding effect on the growth (area increase) of heart cultures from the 8-day chick embryo. Furthermore, experiments in which cultures were immersed in 1.0 per cent thiourea solutions for periods varying from 5 minutes to 2 hours showed that thiourea arrests mitosis at metaphase, inhibits spindle formation, and causes the chromosomes to be dispersed in the cytoplasm. The effects of the treatment, however, are reversible, for normal mitotic activity reappeared after the cultures were washed in Ringer's solution. Treatment with thiourea for 2 to 24 hours results in severe damage (*Rosin, Tenenbaum, and Doljanski, 1951*).

In cultures of heart fibroblasts, colchicine exerts its well-known adverse effect on mitosis (*Pires Soares, 1944a*). Weak solutions inhibit mitosis at metaphase, stronger ones inhibit it at prophase. The nuclei become nearly pycnotic, and the chromosomes are swollen and more or less massed together (*Verne and Vilter, 1940a, 1940b*). Many compounds related to colchicine are inhibitory to various degrees (*Lettre and Fernholz, 1943; Holeckova, 1951*). It has been suggested that the effect of colchicine and other alkaloids on mitosis depends upon the presence of the stilbamine group (alpha, beta-diphenylethylamine) in the molecule (*Lettre and Albrecht, 1944; Lettre and Delitzsch, 1944*). Not all alkaloids possessing this group are mitotic poisons, however (*Lettre and Delitzsch, 1944*). Colchicine also affects the cytoplasm of cultured heart fibroblasts; the cells become independent of each other and then send out many very active pseudopodia (*Miszurski and Doljanski, 1947*).

The effect of cortisone and 17-hydroxycorticosterone on the growth (area increase) of chick heart fibroblast cultures is moderately inhibitory (*Barber and Delaunay, 1951; Kaufman, Mason, and Kinney, 1953*). Cortisone acetate, on the other hand, seems to be without influence (*Baldridge, Kligman,*



*Lipnik, and Pillsbury, 1951; Mancini and Sacerdote de Lustig, 1951; Steen, 1951; Kaufman, Mason, and Kinney, 1953).*

Diethylstilbestrol, 40 to 100 gamma ( $\gamma$ )/cc., and sodium estradiol phosphate, 60 to 80 gamma ( $\gamma$ )/cc., inhibit mitosis in cultured chick heart fibroblasts (*Lettre, 1943*).

## THE FUNCTIONAL DEVELOPMENT OF THE HEART

The cardiac cycle consists of the same sequence of events in the avian and the mammalian heart. The auricles, full of blood, contract and eject their contents through the open auriculoventricular valves into the dilated ventricles. As the auricles relax and begin to fill again with venous blood, the ventricles contract, forcing the auriculoventricular valves to close and the semilunar valves to open. When the ventricles have emptied, the semilunar valves close, the auriculoventricular valves open, the ventricles relax, and blood begins to flow into them from the still expanded auricles. The next auricular systole, initiating another cardiac cycle, occurs near the end of the period of ventricular diastole.

The functioning of the heart is a manifestation of certain characteristic properties of myocardial tissue. The primary property, of course, is the ability to contract in response to an appropriate stimulus; hence it is apparent that cardiac muscle exhibits contractility and excitability. The orderly contraction of all parts of the heart implies also the capacity of cardiac muscle to conduct excitatory impulses. The most outstanding physiological feature of heart tissue, however, is autonomy, for the stimulus that initiates each cardiac cycle is not neural but originates spontaneously within the heart itself, in the sinoatrial node, or "pacemaker." These stimuli are discharged with a rhythmicity which is another physiological peculiarity of the heart.

The heart is the first internal organ to function during embryonic development. Furthermore, it is unique in that it becomes physiologically active before its morphogenesis is completed, its tissue differentiated, or its nerve supply present (*Laveran, 1878*). Although its initial contractions are not identical with those of the adult bird's heart, they are expressions of the same properties that reside in fully differentiated cardiac muscle. In fact, the embryonic heart exhibits a surprising physiological maturity from the beginning, although, of course, it undergoes perceptible functional evolution during incubation.

### Autonomous Contractility of Embryonic Heart Tissue

Embryonic cardiac tissue reveals its inherent capacity for autonomous, rhythmic contraction early in development when the heart begins to beat without the intervention of external stimulation. Each successively added



segment of the heart possesses the property of contractility to a greater degree than the preceding segment; with the result that the tubular heart, as it elongates, exhibits a continually increasing pulsation frequency.

The functional autonomy of embryonic myocardial tissue is further demonstrated by the well-known ability of the entire or fragmented embryonic chick heart to continue to beat when excised and held or cultivated *in vitro* under the proper conditions (Schenk, 1867; Fano, 1885; Burrows, 1910, 1911; Shipley, 1916; Lewis, 1926; Olivo, 1928a; and many others). Pulsation has been seen in a culture of the chick embryo's heart 158 days after explantation (Hogue, 1937). If the cardiac primordia of the chick are explanted to culture media at any time before contraction begins, the tissue not only differentiates histologically but also evinces contractile activity (Olivo, 1928a, 1928e, 1928f).

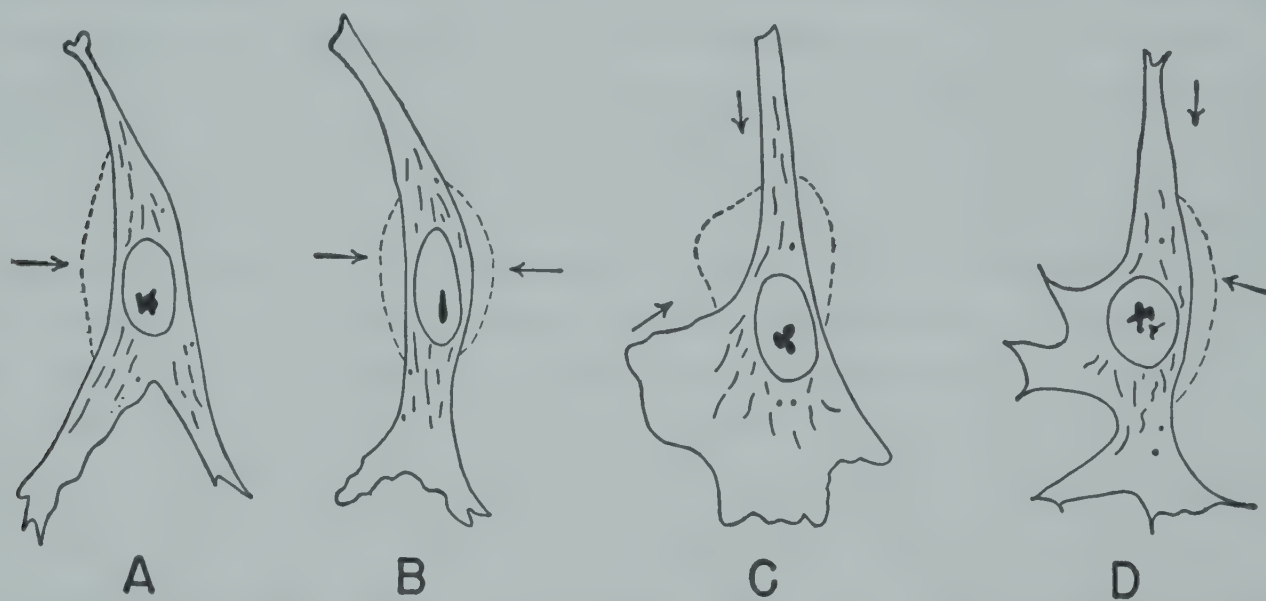


Fig. 290. Changes in the shape of a single heart muscle cell undergoing rhythmical contraction *in vitro*. (Redrawn with modifications after Lewis, 1920.)

This cell was contracting at the rate of 115 beats per minutes in a 48-hour culture of heart muscle from a 4-day chick embryo. Drawings A through D were made at intervals of 15 minutes. Dotted lines indicate pulsating areas of the cell, and arrows indicate direction of motion. All  $\times 350$ .

The property of autonomous contractility apparently resides in the individual myocardial cell (Lewis, 1920). Isolated cells from cultures of embryonic chick heart tissue have been seen to undergo a change in shape as they contract (Fig. 290). This change involves the entire cell even when myofibrils are confined to a small portion of it; therefore it appears that the sarcoplasm and the myofibrils act synchronously to produce contraction (Renyi and Hogue, 1938). Olivo (1929) suggested that autonomous function is principally a property of the sarcoplasm. Cultured embryonic chick myocardial cells may continue to exhibit purely sarcoplasmic contraction for some time after all myofibrils have disappeared (Bucciante, 1927b; Olivo, 1929), although pulsation eventually ceases. Also, explants of 17-day ventricles, which do not possess the capacity for autonomous contraction,



pulsate spontaneously (though temporarily) as soon as myofibrillar dedifferentiation has occurred, apparently because contractility is still latent in the sarcoplasm (Olivo, 1926*b*, 1926*c*, 1929).

Both *in vivo* and *in vitro*, the expression of the capacity for autonomous contraction is subject to the modifying influence of such factors as temperature and the chemical environment.

### *The Initiation of the Heartbeat*

In the nineteenth century, it was generally agreed that the chick embryo's heart begins to contract after 36 to 40 hours of incubation (Prévost and Dumas, 1824; Thomson, 1830; Prévost and Lebert, 1844; Preyer, 1885), rather than after 45 to 51 hours, as Haller (1754, 1756, 1758) had previously stated. It now appears that cardiac activity starts even earlier, at the 9-somite (Olivo, 1925*a*, 1930) to 10-somite stage (Rückert and Mollier, 1906; Sabin, 1917*b*, 1920; Johnstone, 1925; Patten and Kramer, 1933). In fact, the chick's heart sometimes beats at the 7-somite stage (Olivo, 1924*b*, 1925*a*), in which case the two cardiac primordia, not yet entirely fused, may contract asynchronously. Independent contraction of the two sides of the heart has also been observed in diplocardiac (double-hearted) embryos (Dareste, 1876; Fano, 1885; Féré, 1895; Waddington, 1932; Szepsenwol, 1933*b*, 1934; Waddington and Cohen, 1936). In the duck (*Anas platyrhynchos*) embryo, the heart apparently begins to function at the 13- or 14-somite stage, or as soon as the paired endothelial tubes fuse into a single tube (Weber, 1902*e*; Boerner-Patzelt, 1931).

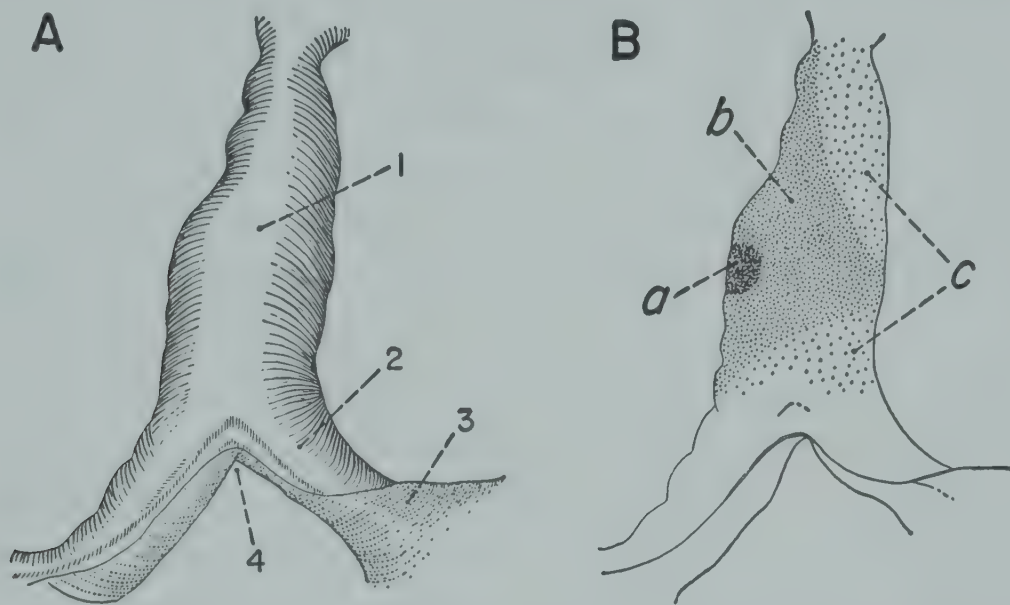
Contractile activity usually begins in a small area along the convex right margin of the newly formed cardiac tube (Sabin, 1920; Johnstone, 1925; Patten and Kramer, 1933), which represents only the bulboventricular portion of the heart (Fig. 291); however, it is possible for it to start on the left side of the heart (Olivo, 1924*a*). The earliest contractions are mere flickers. Johnstone (1925) stated that they are localized about three eighths of the distance from the caudal to the cephalic end of the heart, but Patten and Kramer (1933) found that they do not always appear in the same spot. Several sporadic twitches occur in succession and are followed by a pause, often of 5 minutes' duration. Within a few minutes after the beginning of this irregular local activity, the entire right side of the ventricle, or most of it, becomes involved in contraction. This region now supersedes the venous extremity of the heart as the portion exhibiting the highest metabolic rate, as shown by susceptibility to toxic solutions (Hyman, 1927*b*) and by the rapidity of Janus green reduction (Rulon, 1935).

In about an hour (Patten and Kramer, 1933), the contracting area spreads laterally to include a point on the left side of the heart (cf. Fig. 291) about one third of the distance from the venous to the arterial end (Johnstone, 1925). Soon the entire left side is contracting synchronously



with the right side. The heartbeat at this time (the 11-somite stage) consists merely of a simultaneous movement of the walls of the ventricle toward each other. Five to ten slow contractions are followed by a rest period 1 to 3 minutes in length (*Patten and Kramer, 1933*). Ligature (*Johnstone, 1925*) or transection (*Patten and Kramer, 1933*) of the heart at the caudal end of the ventricle does not cause pulsation to cease; the heartbeat, therefore, originates in the ventricle at this time.

The atrium begins to contract as soon as it becomes recognizable as part of the heart, that is, at the 12- to 13-somite stage. Very shortly afterward, the heartbeat changes markedly in character. It becomes peristaltic, sweep-



**Fig. 291.** Site of initial contractions in the heart of the developing chick. (Redrawn with modifications after *Johnstone, 1925*.)

A, drawing of the heart of the 10-somite chick embryo, ventral view, showing the initial bulging of the right side of the primitive ventricle; B, schematic outline of heart shown in A. The dark area, *a*, on the bulging greater curvature represents the region in which the first pulsations usually begin. From area *a*, the beating portion spreads gradually along the entire right border of the heart and across the ventricle to the left border to include area *b*. The pulsations finally extend over the whole of the primitive ventricle, encompassing area *c*. Both  $\times 50$ .

1, primitive ventricle; 2, primitive sinus venosus; 3, omphalomesenteric vein; 4, anterior intestinal portal.

ing in the form of a contraction wave from the atrial to the bulbar end of the heart, that is, posteroanteriorly (*Patten and Kramer, 1933*).

At the 16- to 17-somite stage, the contractions of the heart become sufficiently strong to start the circulation of blood. The nature of the heartbeat is not modified at this time, except for slight passive dilatation of the heart wall due to the accumulation of blood ahead of each contraction wave (*Patten and Kramer, 1933*). At first, the blood cells are propelled in a jerky manner. Between each heartbeat, they pause momentarily or even retrogress slightly. This "backlash" of the blood current is especially noticeable in the veins and arteries leading into and out of the heart (*Patten, Kramer, and Barry, 1948*).



Early in the third day of incubation, the blood cells begin to pass more evenly and smoothly through the heart and large vessels. This change is the result of valvular action exerted by accumulations of cardiac jelly under the endothelium at the points of transition from sinus venosus to atrium and from ventricle to truncus arteriosus.

Meantime, between the stages of 20 and 25 somites, the sinus venosus is established. Like the atrium, it begins to contract as soon as it has differentiated and received its epimyocardial investment (*Patten and Kramer, 1933*). The point where contraction originates in the sinus, however, has been seen to migrate back and forth from caudal to cephalic sinus levels (*Barry, 1942*).

It is apparent, from the foregoing, that the heartbeat is initiated at a continually more posterior level as the heart primordia fuse, since the contraction wave always begins in the most recently formed (that is, caudalmost) portion of the heart.

### *The Rate of Contraction*

In birds, heart frequency varies inversely with body weight and directly with metabolic rate, as it does in mammals. In general, birds and mammals of comparable body weight exhibit more or less similar heart rates (as well as metabolic rates), but the cardiac tempo of birds is somewhat faster. It is probable, also, that the heartbeat is subject to less control by the vagus nerve in birds than in mammals. The vagus not only slows the heart when necessary but also exercises a constant restraint on it. The degree of vagal influence varies in different avian species and is most pronounced in birds with relatively large hearts. It is very slight in the chicken (*Stübel, 1910; Bogue, 1932*), the vulture, *Aegypius monachus*, the rook, *Corvus frugilegus*, the hooded crow, *Corvus cornix*, and the jackdaw, *Corvus monedula* (*Stübel, 1910*), moderate or variable in the buzzard, *Buteo buteo*, and the kestrel, *Falco tinnunculus* (*Stübel, 1910*), and readily demonstrable in the duck, *Anas platyrhynchos* (*Stübel, 1910; Bogue, 1932*), the gull, *Larus canus*, the kite, *Milvus* sp., and the hawk, *Accipiter* sp. (*Stübel, 1910*).

The heart rate of the avian embryo is not stabilized until long after contraction begins, and, at all ages, it varies greatly among different individuals (*Pickering, 1893a; and many others*). Nevertheless, pulsation frequency exhibits certain definite trends during incubation. First, there is a period of acceleration, rapid initially and then more gradual, until a maximum frequency is attained. In the chicken and probably other birds, the increase in heart rate is followed by a decrease before hatching and a further increase afterward. This characteristic functional behavior contrasts decidedly with the continual prenatal and postnatal deceleration typical of the mammalian heart and, in the chicken, may in part reflect the relative lack of vagal control (*Bogue, 1932*).



The initial rapid rise in heart frequency is associated with the establishment of a primitive pacemaker and reflects the different degrees of autonomy inherent in the various regions of the tubular heart. The causes of the subsequent period of continued though slower acceleration are not known, but they may be of circulatory origin. The final check placed upon the heart is possibly due to the belated imposition of neural control. Changes in the embryonic heart rate are not correlated with sex or with the basal metabolic rate (Bogue, 1932).

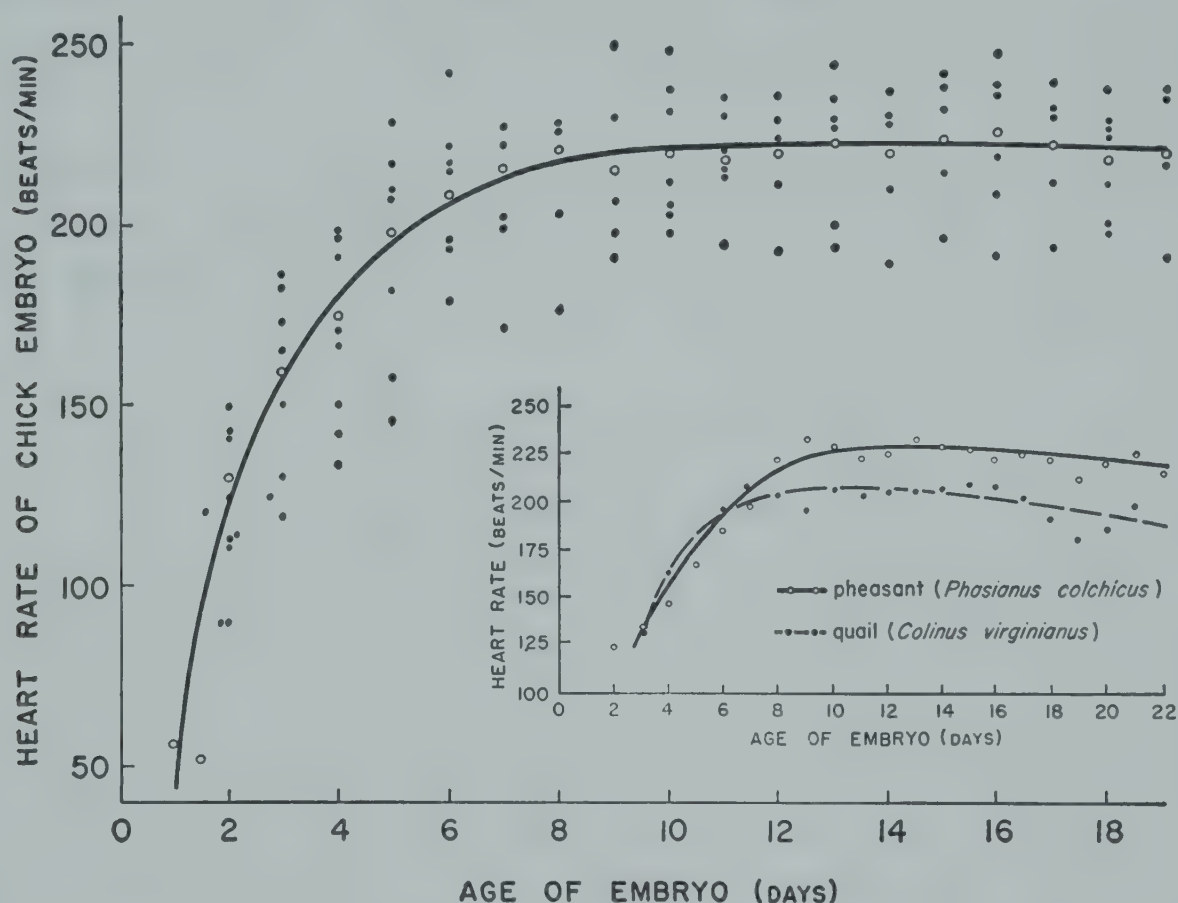


Fig. 292. Changes in the rate of heartbeat in the developing chick embryo. Small dots represent the variations of rate at each age; large dots show the averages of these. (Based on the data of Wernicke, 1876; Féré, 1895; Cohn and Wile, 1925a; Cohn and Porosowsky, 1928; Romanoff, 1929; Andersen, 1932; Bogue, 1932, 1933; Kuo, 1933; Romanoff and Sochen, 1936; Barry, 1940; Alexander and Glaser, 1941.)

*Insert:* comparison of heartbeat rates of two other gallinaceous birds, the ring-necked pheasant, *Phasianus colchicus*, and the bobwhite quail, *Colinus virginianus*. (Based on the data of Romanoff, 1944a.)

As Fig. 292 shows, the period of most rapid acceleration of the chick's heartbeat terminates at the end of the second day, and from this time until the fifth day the rate of increase is slightly lower (Wernicke, 1876; Féré, 1895). Incomplete observations on the starling (*Sturnus vulgaris*), the European redstart (*Phoenicurus phoenicurus*), and the linnet (*Acanthis cannabina*) indicate that there is a comparable increase in cardiac tempo early in the incubation periods of these altricial birds (Groebels, 1933). The chick's heart continues to accelerate, but at a considerably more gradual rate, until the eighth or tenth day (Preyer, 1885; Bogue, 1932), when the maximum frequency is reached (Romanoff, 1929, 1944a; Kuo,



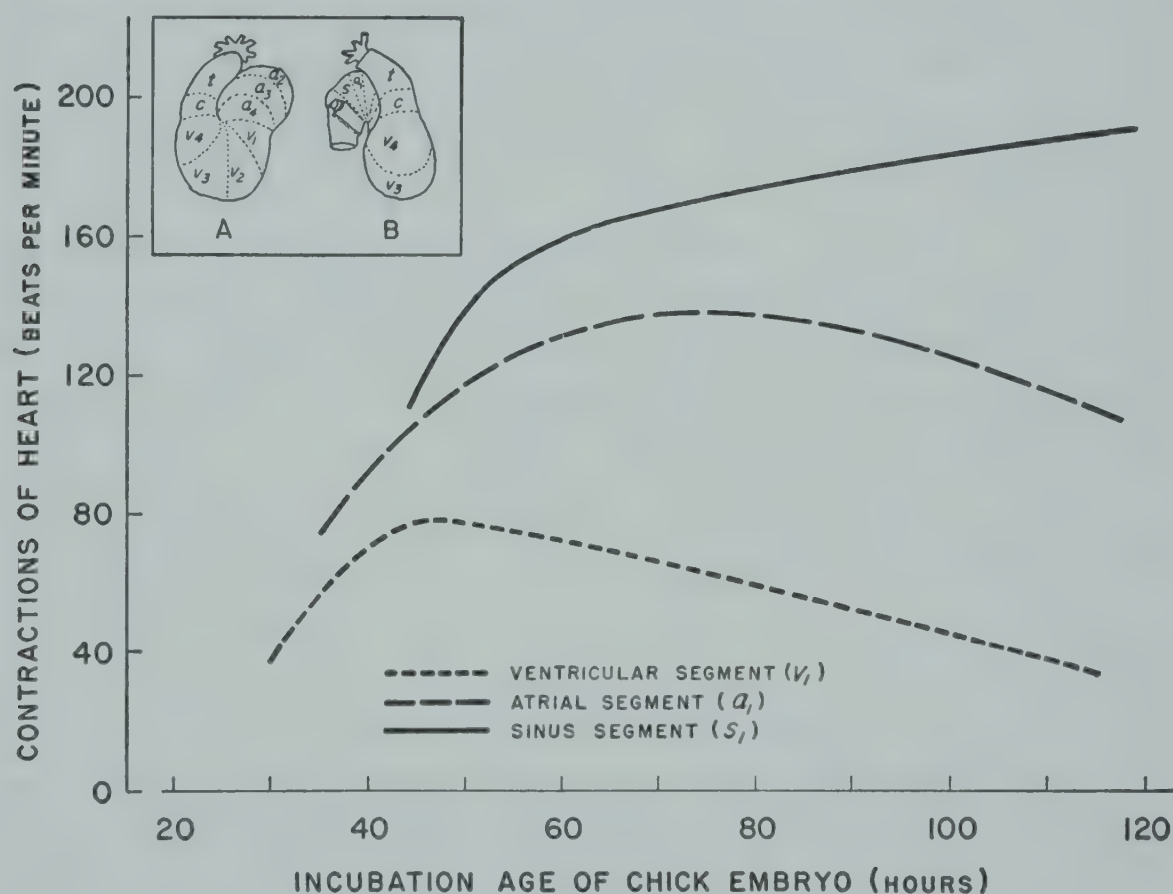
1933; Romanoff and Sochen, 1936; Barry, 1940). It is possible, however, that acceleration continues until the thirteenth or sixteenth day (Cohn and Wile, 1925a; Cohn and Porosowsky, 1928; Bogue, 1932; Andersen, 1932). In the quail (*Colinus virginianus*) and the ring-necked pheasant (*Phasianus colchicus*), the peak frequency is observed after the passage of about 40 per cent of the total incubation period (Romanoff, 1944a). Once the maximum rate is attained, a slight deceleration sets in (Cohn and Wile, 1925a; Romanoff, 1929, 1944a; Kuo, 1933).

The initial period of most rapid acceleration coincides with the time during which the primitive chambers of the tubular heart differentiate and start to pulsate. At first, when contraction is limited to the future bulbo-ventricular region, the pulsation rate is slow and variable. Between the thirtieth and the forty-third incubation hour, the chick's heart accelerates greatly as it is lengthened by the addition first of the atrium (Patten and Kramer, 1933; Barry, 1940) and then of the sinus venosus (Barry, 1940). It can be inferred, therefore, that each successively added segment of cardiac tissue has a higher intrinsic contraction rate, or greater degree of autonomy, than the portion immediately cephalic to it, and that the heart becomes functionally dominated by these segments in turn (Barry, 1940, 1942). Studies of gradients in susceptibility to toxic solutions have shown that the most recently added segment of the 16- to 20-somite heart is most susceptible and hence is the region where the metabolic rate is highest (Hyman, 1927b).

The existence of intrinsic regional differences in rate has been demonstrated simply by interrupting the continuity between the various portions of the heart (*in situ* or excised) by such means as ligature or transection (Fano and Badano, 1890a; Pickering, 1893a; Johnstone, 1924; Lewis, 1924; Patten and Kramer, 1933; Paff, 1935). Division of the 2-day heart into bulbus, ventricle, and sinoatrium reveals that each portion has its characteristic tempo. The sinoatrial beat is most rapid, and approximates that of the intact heart; the ventricular rate is only one third to one half as fast; and bulbar contraction is very slow and irregular, or absent entirely (Lewis, 1924). The fact that the sinoatrium controls cardiac rate at the end of the second day has been shown experimentally by transecting the 48-hour heart at the atrioventricular junction and leaving the two segments in apposition. They are soon united through the growth of new tissue. The contraction rate of the bulboventricular segment then increases until it equals that of the sinoatrial segment, and the entire heart again exhibits a unified rhythm (Paff, 1935). The dominance of the sinoatrial region has also been demonstrated by transferring this segment to the bulbar end of the bulboventricular segment, whereupon the contraction wave, which still originates in the sino-atrium, is reversed in direction and proceeds from the atrial to the venous end of the heart (Paff, 1936). Again, reversal



of the heartbeat may occur when the dominance of the sinoatrium is suppressed through the depression of oxidative processes within it, for initiation of the contraction wave may then be restored to more cephalic levels where the metabolic rate is lower. Reversed contraction has been observed, for example, after the application of hydrocyanic acid to the 3-day heart (*Pickering, 1893a*) as well as after an artificial increase in the carbon dioxide content of the vitelline blood entering the 2-day heart (*Sangvichien, 1952*). When the 2- to 6-day heartbeat is slowed by low temperature, stimulation of the ventricle or bulbus by a short exposure to ultraviolet point radiation reverses the direction of contraction (*Hinrichs and Warwick, 1931*).



*Fig. 293.* Curves showing the changes in the respective intrinsic contraction rates of the caudalmost ventricular, atrial, and sinus segments of the chick embryo's heart from the time each segment starts to beat up to the middle of the fifth incubation day. (Redrawn with modifications after Barry, 1942.)

*Insert:* the actual location of various heart segments. A, ventral view; B, dextral view.

c, the conus (the tapering portion of myocardial tube between the ventricle and the future site of the semilunar valve); t, truncus.

It has also been shown that there is an activity gradient within the various cardiac regions, as well as between them, for division of each differentiated portion of the 30- to 120-hour heart into several transverse segments reveals that the caudalmost segment of every region is the most rapidly beating one (*Fig. 293*). Thus the intracameral gradient, like the intercameral, increases in the direction in which the myocardium is added, that is, toward the caudal end of the heart. It follows, therefore, that the caudalmost part of the sinus venosus possesses the highest inherent con-



traction rate. Fluctuations in heart frequency can be detected as the pacemaking function migrates back and forth in the sinus. Up to the end of the fifth day, the pulsation frequency of the caudalmost sinus segment accelerates at a rate strikingly parallel to that shown by the beat of the intact heart (*Barry, 1942*). Transection and explantation experiments on older hearts show that the tempo of the isolated right auricle (exclusive of its lateral portion, but presumably inclusive of the sinus) approximates that of the entire heart after the fifth to seventh day (*Cohn and Wile, 1925b; Bisceglie, 1929*).

The intrinsic contraction rate of every level of the myocardium, except the caudalmost, undergoes an increase and then, by the end of the chick's fourth incubation day, starts to diminish (cf. Fig. 293). The ventricular rate reaches its maximum at the end of the second day (*Lewis, 1924; Barry, 1942*); the auricular, 24 hours later (*Barry, 1942*). The subsequent decline is symptomatic of the gradual loss of autonomy observed by *Fano (1885)*. The decreasing contractile capacity of the ventricle is indicated by the progressively shorter duration of pulsation in cultured ventricular fragments taken from increasingly older embryos (*Olivo, 1929*); see the accompanying table (*Nordmann and Rüther, 1931*). The isolated ventricle can no longer pulsate at all after the tenth or thirteenth day (*Bottazzi, 1896; Burrows, 1912; Cohn and Wile, 1925b; Olivo, 1926b, 1926c, 1929; Nordmann and Rüther, 1931*), and the atria begin to fail in this respect after the tenth day (*Cohn and Wile, 1925b; Nordmann and Rüther, 1931*). The proportion of pulsating cultures that can be obtained from the heart therefore drops as the age of the embryo increases. It should be noted here that the contraction rates of cultured heart fragments are extremely variable (*Hogue, 1937, and many others*).

Age of Culture (days)	Percentage of Pulsating Heart Cultures from Chick Embryos of Various Ages		
	5-7 Days (per cent)	9 Days (per cent)	11-15 Days (per cent)
1	84	40	20
2	73	30	10
3	66	16	0
4	53	12	
5	46	7	
6	34	2	
7	27		
8	25		
9	12		
10	7		
11	3		

According to *Barry (1942)*, each portion of the myocardium can be said to pass through a series of functional phases. A phase in which autonomy



has not yet developed is succeeded by one in which pulsation begins spontaneously and shows an initially rapid acceleration to a rate higher than that of any previously formed levels of the heart. A third phase then follows during which there is deceleration of the inherent contraction rate, although the tissue now beats synchronously with a new, more caudal pacemaker at a rate higher than its own. Lastly, the atrial and ventricular myocardium loses its capacity for independent contraction, although it is still able to respond to stimuli; the caudalmost (pacemaking) segment alone retains its autonomy (cf. Fig. 293).

The continuing but more gradual acceleration of the heartbeat after the end of the chick's second day of incubation has been tentatively explained on the basis of the effect of factors of circulatory origin on the inherent contraction rate of the pacemaker (*Alexander and Glaser, 1941; Barry, 1941*). The exact mechanisms involved are not entirely clear; however, Alexander and Glaser (1941) found that pulsation frequency can be increased experimentally by constriction of the heart or its afferent veins or by dilation of its efferent arteries. Accordingly, they accounted for the acceleration in cardiac tempo from the third to the ninth day on the basis of chronic and cumulative underdistension of the heart—a condition supposedly due, in turn, to the development of peripheral arteries and blood at a faster rate than that at which veins develop to return blood to the heart. On the other hand, Barry (1941), like Wernicke (1876), observed that complete or partial exsanguination of the heart causes a decrease in contraction rate, the decrease generally varying directly with the age of the embryo (40 to 140 hours). Resumption of blood flow after partial exsanguination often restores the original tempo. It was concluded (*Barry, 1941*) that blood pressure within the embryonic heart has an important bearing on the rate of myocardial contraction. Assuming that the intrinsic contraction rate of the pacemaking tissue is a direct function of the tension of the myocardial wall, it follows that the prolonged acceleration of cardiac frequency is the result of the rise in blood pressure during incubation.

The increase in the chick's heartbeat during incubation has also been viewed simply as the result of regulation of the circulation by a heart of continually smaller relative weight in terms of total body weight. When the capacity or the stroke volume of the heart is considered instead of its weight, the decrease in proportional size is even more marked and the inverse relationship to contraction rate more apparent (*Andersen, 1932*). It may be noted that Kuo (1933) found the period of maximum body activity in the embryo chick to coincide with the period in which the heart attains its highest frequency.

It is problematical to what extent the eventual stabilization of the chick embryo's heartbeat at a rate close to its maximum is due to the "trapping" of the heart by the vagus nerve. Pickering (1896) found that the passage



of a weak interrupted (induced) current through the heart causes tonic contraction before the sixth to eighth day but inhibition of cardiac function after that time. (He was unable to produce inhibition with a constant current.) His numerous experiments indicated that the strength of interrupted current needed to inhibit the heartbeat is subject to influences that modify the metabolism of cardiac muscle (temperature, oxygen and carbon dioxide concentration) or its tonus (drugs such as caffeine, digitalis, and veratrine); but he also considered it significant that electrical inhibition is affected by the antagonistic action of muscarine (heart-inhibiting) and atropine (vagus-blocking) and becomes possible at the time when the action of these drugs is first demonstrable (*Pickering, 1895b*). *Pickering (1896)* concluded, therefore, that neural processes begin at the 6- to 8-day stage to assert their dominance over processes confined to the contractile tissue.

On the other hand, it has been observed that direct excitation of the vagus trunks produces no regular retardation in the chick's heart rate until after hatching, although a vagal effect can sometimes be elicited on the last day or two of incubation (*Bottazzi, 1896; Iankovskaia, 1949*). In fact, the injection of atropine has been found to have no significant accelerating effect on the pulse rate of young hatched chicks (age not specified); however, it causes a definite increase in the heartbeat of young ducklings, *Anas platyrhynchos* (*Bogue, 1932*).

In the chick, the only clear-cut postembryonic change in cardiac frequency occurs during the first 12 hours after hatching. At the end of this time, the heart rate has increased to approximately the average adult value. Observations are made difficult by the extreme variability in heart frequency in each individual (*Bogue, 1932*). In nestling altricial birds, the heart rate is subject to the effect of external temperature until homoiothermy is established; but perhaps it is significant that, at the point of thermal neutrality (the temperature at which the heartbeat is slowest throughout adult life), the pulse of the nestling house wren (*Troglodytes aedon*) is the same on the first day after hatching as later (*Odum, 1941*).

**Output and work of the chick embryo's heart.** From the data of *Bogue (1932)* for the changes in heart rate during incubation, and from estimated values for stroke volume over the same period, *Hughes (1949)* calculated

Age of Embryo (days)	Heart Output (ml/min)	Work Done by Heart (ergs/sec)
2.5	0.0036	
4.0		3.5
5.0	0.12	
6.0		34.0
7.0		69.0
12.0	4.8	
17.0	6.3	



output per minute for the heart of the chick embryo of various ages; the resulting figures are given herewith. Estimations of the work done by the heart (also given in the accompanying table) were then made from the data for cardiac output and arterial pressure (*Hill and Azuma, 1927; Hughes, 1942*) which is low (about 2.0 cm. of water) on the end of the second incubation day, rises gradually (to approximately 16 cm. of water) until the twelfth day, and then increases rapidly until the eighteenth day, when it reaches a stationary value (about 35 cm. of water).

### *The Influence of Temperature*

The heart of the avian embryo is able to beat only within a relatively narrow temperature range, yet its resistance to prolonged exposures to heat and cold is quite remarkable. Within the thermal limits that permit contractile activity, cardiac pulsation frequency is determined to a large extent by temperature.

**Functional Capacity at Abnormal Temperatures.** The heart of the avian embryo stops beating if it is exposed to temperatures outside a certain limited range. Loss of functional capacity may be temporary or permanent, depending upon conditions.

According to Pickering (1893a), the effect of cooling the chick embryo's heart is to weaken the systoles and expand the whole heart somewhat. Overheating likewise diminishes the length of systoles and causes imperfect diastoles. Weber (1846) stated that heat tetanus does not occur; Pickering (1893a) interpreted the shortened systoles that precede cessation of function as being due to tonic or idiomuscular contraction. The heart usually stops in diastole, both at low temperatures (*Wernicke, 1876*) and at high temperatures (*Pickering, 1893a*).

The temperature range permitting cardiac function increases somewhat with age, and it may therefore be concluded that the heart's susceptibility to abnormal temperatures decreases as the embryo grows older. Early investigators indicated that the chick's heart can beat between 10° C. (*Preyer, 1885*) and 50° C. (*Weber, 1846; Preyer, 1885*). Schenk (1867) placed the temperature limits for the 3-day chick's heart at 23° C. and 41° C., and Eckhard (1867) specified that 41° to 42° C. was the upper limit for the 8- to 10-day heart also. Later research has revealed more clearly the relationship between embryonic age and the resistance of the heart to elevated and reduced temperatures. When deviation from the normal incubation temperature (37.5° C.) is accomplished in a series of 3-degree changes applied every 10 minutes, the limits within which the chick embryo's heart (*in situ*) is able to beat are extended during incubation in the manner shown in the accompanying tabulation (*Romanoff and Sochen, 1936*). Lewis (1924) remarked that the excised 2- or 3-day chick heart sometimes does not cease beating until the gradually rising temperature reaches 50° C.



The 3.5-day duck (*Anas platyrhynchos*) embryo's heart (*in situ*, but after removal of the embryo from the egg) functions over the same range as the 3-day chick embryo's heart (*Inukai, 1925*).

Age (days)	Lower Limit (°C.)	Upper Limit (°C.)
3	13.5	46.5
8	10.5	49.5
13	7.5	52.5
18	4.5	61.5

During the course of incubation, the resistance of the heart to suddenly applied abnormal temperatures apparently changes somewhat differently from its resistance to such temperatures applied gradually. The heart of the 18-day chick embryo beats three times as long at 16.5° C. as does that of the 3-day embryo, but at 58.5° C. the older heart pulsates only 60 per cent as long as the younger heart (*Romanoff and Sochen, 1936*). It appears, therefore, that the embryonic heart, as it grows more mature, becomes better able to withstand very rapid chilling and less able to endure very rapid overheating.

The excised heart can function for extended periods of time at abnormal temperatures. The data of *Bucciante (1927a)* are given herewith to show how the maximum duration of contractility varies according to the temperature at which fragments of the 7- to 8-day chick embryo's heart are held in unrenewed plasma. Like *Lambert and Hanes (1913)*, *Bucciante (1927a)* found that the average duration of contractility at 38° C. or 39° C. to 44° C. is shorter than at 25° C. (at which it is 12 to 18 days), probably because at 25° C. the metabolic rate is lower than at the higher temperatures. Yet the unfavorable effects of cold do not appear as quickly as at still lower temperatures.

Temperature (°C.)	Maximum Duration of Pulsation (days)
25-26	29
21	12
18	10
12-13	6.5

It has been known for many years that cessation of the heartbeat when the heart is heated or cooled does not necessarily prevent function from being resumed when the temperature returns to a more favorable level. *Schenk (1867)* observed that a 3-day chick embryo's heart, paralyzed by exposure to 8° C. or 41° C., began to contract again at 32° to 34° C.; *Cleland (1877)* noted that a heart that had stopped at room temperature



started to beat once more when the egg was held close to a lighted lamp; Fano (1885) described similar results upon transferring an excised 3-day heart (in sodium chloride solution) from 16° C. to 38° C.; and Bucciante (1927a) observed that fragments of the 7- to 8-day chick embryo's heart (in plasma) ceased pulsating at 0° C. but again exhibited contractility when the temperature was elevated to 18° C. The excised heart of the young embryo is even able to withstand immersion in liquid nitrogen (−195° C.) for a limited time if it has been partially dehydrated in ethylene glycol, dehydration presumably eliminating the formation of large ice crystals. Excised hearts of 2- and 3-day embryos subjected to this treatment may start to beat within 2 to 11 minutes after being placed in Tyrode's solution at 40° C. and may continue to pulsate for 30 to 80 minutes (Campbell, 1950). When left *in situ*, the partially dehydrated 36- to 48-hour heart may be held at −195° C. for an hour and subsequently may beat for 30 minutes to 20 hours at 40° C., although not without showing evidence of injury (Gonzales and Luyet, 1950). Bucciante (1927a) pointed out, however, that elevation of temperature cannot induce a renewal of function or of any other vital activity in 7- to 8-day heart fragments that have spontaneously come to a halt after pulsating for several days in plasma at temperatures of 12° C. to 25° C. He therefore concluded that the cardiac muscle cell retains its contractility when vitality is at a minimum and loses its functional capacity only at the moment of death.

**Thermal Affects on the Heart Rate.** The heart rate of the avian embryo is a direct function of environmental temperature (Harvey, 1651; Schenk, 1867; Cleland, 1877; Preyer, 1885; Pickering, 1893a, 1893b). At every age, any increase in temperature (over a certain range) produces an acceleration of the heartbeat. Abrupt exposure to low (16.5° C.) and high (58.5° C.) temperatures resulted in changes in the rate and duration of heartbeat of the developing embryo as shown in Table 13. These relationships are apparent not only when the heart is left *in situ* but also after it has been excised (Lewis, 1924, 1929). However, the influence of temperature is not exactly the same at all incubation ages, nor are temperature-induced changes in heart rate of equal magnitude at every temperature level.

In general, the average change in heart rate with each degree of temperature change decreases throughout the incubation period. Expressed mathematically, the average temperature coefficient ( $Q_{10}$ ) drops from slightly more than 2 on the third, fourth, and fifth days to 1.5 on the eighteenth day (Cohn and Porosowsky, 1928)—individual values varying, however, between 1.5 and 4.3 (Murray and Porosowsky, 1926). It may be noted, however, that there is one record of low initial values (of the order of 1.2 to 1.4) during the second and third days, with an increase to 2.9 on the seventh day and a subsequent decrease to 2.0 on the ninth day, after which observations were not made (Cesana, 1912). A progressive decline



# Changes in Rate and Duration of Heartbeat of the 3-, 8-, 13-, and 18-Day-Old Chicken Embryos Under Exposures to Low or High Temperatures \*

\* From the original data of Romanoff and Sochen (1936), and Romanoff (*unpublished*).



in the average temperature characteristic (critical thermal increment), calculated by a modification of the Arrhenius formula (*Arrhenius, 1915*)

$$(\mu = 4.61 \frac{\log K_2 - \log K_1}{\frac{1}{T_1} - \frac{1}{T_2}}), \text{ where } T_1 \text{ and } T_2 \text{ are absolute temperatures}$$

and  $K_1$  and  $K_2$  are the respective heart rates at those temperatures), bears out the conclusion that sensitivity to temperature diminishes continually from the third day until at least the seventeenth or eighteenth day of incubation (*Murray and Porosowsky, 1926; Cohn and Porosowsky, 1928*). During this period, the average temperature characteristic of the isolated atrium has been found to decrease from 23,500 to 9200 (*Murray and Porosowsky, 1926*) and that of the whole heart *in situ* from 12,400 to 7300 (*Cohn and Porosowsky, 1928*).

Pulsation frequency changes at a (relatively) uniform rate only within certain limited temperature zones. These zones are separated by "critical points" at which the rate of change in cardiac tempo is abruptly altered. Critical points are apparent as "breaks" in the curve of heart rate plotted against temperature (*Romanoff and Sochen, 1936*) and are also revealed by sudden changes in the temperature characteristic (*Parpart and Glaser, 1930*). A low critical point at 20° to 25° C. (Fig. 294) seems to exist for the 3-day chick heart (*Romanoff and Sochen, 1936*) and the 3.5-day duck (*Anas platyrhynchos*) heart (*Inukai, 1925*) but is not demonstrable in older chicks. Below 20° to 25° C., temperature changes have the least effect on the 3-day heartbeat. A second critical point is seen in the vicinity of 34.5° to 36.9° C. at the 3-day stage (cf. Fig. 294) but is found at about 33° C. at the 5-day stage; thereafter (cf. Fig. 294—Insert), it lies between 29° C. and 31° C. (*Parpart and Glaser, 1930; Romanoff and Sochen, 1936*). This shift has been correlated with the innervation of the heart (*Parpart and Glaser, 1930*). A third critical point is found above normal temperature; this point also migrates with age (cf. Fig. 294—Insert) from 43.5° C. at 3 days to 38.5° C. at 18 days. The greatest influence of temperature per unit change is seen between 20° C. and 34.5° C. at the 3-day stage and between the lower limiting temperature and 29° to 33° C. at later stages. The effect of temperature changes over this low range is fairly similar at all ages (*Romanoff and Sochen, 1936*), the value of  $\mu$  being most frequently of the order of 18,000 or 25,000 although sometimes of the order of 14,000 or 16,000 (*Parpart and Glaser, 1930*). In the temperature zone between 29° to 37° C. and 43.5° to 58.5° C., the effect of the temperature gradient not only is more moderate (except, perhaps, at the 3-day stage) but becomes less pronounced with age. In this zone, the value of  $\mu$  is of the order of 8500 (*Parpart and Glaser, 1930*). Above the upper critical point, there is a further reduction in positive response to temperature change; in fact, temperature increments produce a



slight deceleration in heart rate before the upper limiting temperature is reached (Lewis, 1924; Inukai, 1925; Romanoff and Sochen, 1936).

It has been pointed out (Parpart and Glaser, 1930) that the values of  $\mu$  that seem to characterize the chick embryo heart at various ages have also been found in myogenic and neurogenic hearts of other animals. These values—of the order of 8000, 14,000, 16,000, 18,000, 20,000, and 24,000—are encountered in connection with various physicochemical processes. Identical physicochemical mechanisms apparently underlie the rhythm of different types of heart at all stages of development (Parpart and Glaser, 1930).

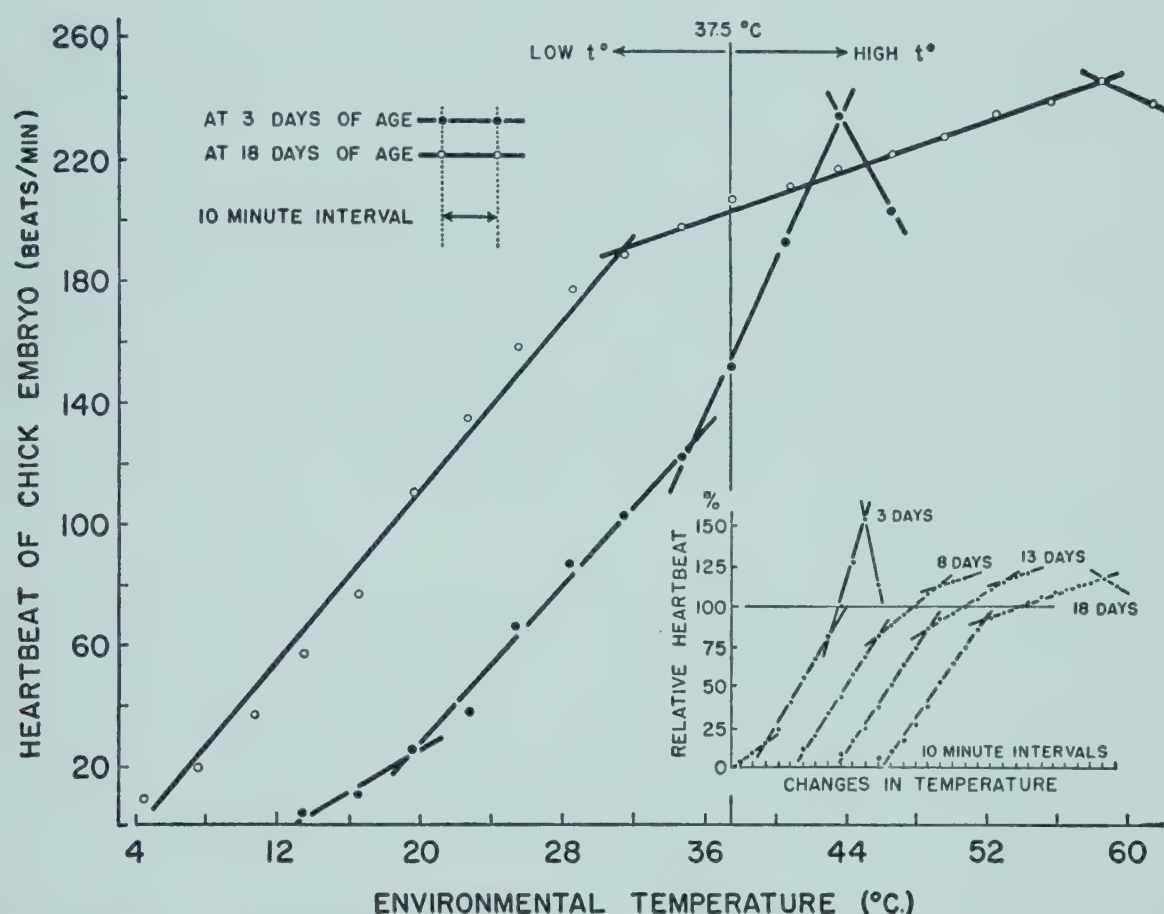


Fig. 294. The effect of gradually applied temperature changes on the heart rate of the chick embryo at the incubation ages of 3, 8, 13, and 18 days. Starting at the normal incubation temperature of  $37.5^{\circ}\text{C}$ ., the temperature was raised or lowered  $3^{\circ}\text{C}$ . every 10 minutes until the heart stopped beating. (Redrawn with modifications after Romanoff and Sochen, 1936.)

*Insert:* relative changes in the rate and duration of heartbeat of the 3-, 8-, 13-, and 18-day-old embryos upon gradual exposure to extreme low and high temperatures.

The isolated ventricle of the 2- to 3-day chick embryo's heart is less markedly and less consistently affected by temperature changes than the isolated atrium (Lewis, 1924). The data of Murray and Porosowsky (1926) suggest the possibility of a slight increase in the sensitivity of the ventricle to temperature with age (between the fifth and the eighth day). Cesana's (1912) observation that the ventricle is more responsive than the atrium does not seem to have been substantiated by later experimental work.

In altricial birds, the heart rate continues to show a thermal response as long after hatching as poikilothermism persists. In the house wren (*Troglodytes*



*dytes aedon*), for example, homoiothermy begins to be established on the sixth postembryonic day. By the twelfth day, the adult reaction to temperature is apparent; that is, an increase in air temperature leads to a decrease, rather than to a rise, in pulse frequency (Odum, 1941). In the black-capped chickadee (*Parus atricapillus*), the transition to homoiothermy occurs between the seventh and ninth days (Odum, 1943). The hatched chick presumably becomes homoiothermic on the fourth day (Lamoreux and Hutt, 1939), but no associated response of the heart has been observed at this time.

### *The Effect of Chemical Environment*

It is well known that the activity of any heart is greatly influenced by its chemical environment. The essential condition for normal function is the presence of certain cations in the correct amount and in balanced ratio. Sodium, calcium, and potassium are the most important of these cations, although others also have an effect. An absolute or relative disproportion between the essential cations affects the rate of cardiac contraction and the conduction of the excitatory impulse from cell to cell and gives rise to various functional irregularities. The impact of variations in the nature and concentration of anions, as compared with cations, is much less pronounced.

Countless experiments attest to the ability of the chick embryo's heart to maintain its functional capacity for protracted periods of time in buffered solutions containing the proper mixture of salts. The effect of the concentration of cations in the perfusion fluid becomes apparent at the 11-somite stage (Olivo, 1924a), or very soon after the heart begins to beat, and has been observed at various ages thereafter.

Since the critical factor is the ratio between the various cations, the behavior of the heart in a solution of a single salt is not too significant, for the modifying effects of the other essential cations are then absent. Nevertheless, the effect of sodium is contrasted with that of calcium and potassium when the chick embryo's heart is perfused with solutions of their respective chlorides. Thus the 3-day embryo's heart continues to beat for about 1.5 hours in 0.75 per cent sodium chloride (Fano, 1885) or 0.9 per cent sodium chloride (Lewis, 1929). In the stronger solution, it may show an initial acceleration of 40 to 100 per cent in rate of beating, followed by a decrease of 25 to 50 per cent, then by atrioventricular block, and finally by gradual weakening and cessation. Hearts cultured in plasma mixed with 0.8 to 1.3 per cent sodium chloride may show various types of heart block and other irregularities, and frequency is reduced in the higher concentrations (Murray, 1935a). In pure potassium chloride (0.96 to 1.0 per cent), however, the heart stops beating almost immediately (Bisceglie and Bucciardi, 1929; Murray, 1938a), and the 3-day heart never pulsates for more



than 3 minutes in pure 0.95 per cent calcium chloride (*Murray, 1938a*). Barium chloride, in the presence of sodium chloride, is a cardiac depressant (*Pickering, 1893a; Sacerdote de Lustig, 1942*), although its effect largely disappears when the temperature deviates in either direction from 37.5° C. (*Pickering, 1893a*) and, like the effects of other cations, can usually be removed by a bath of Ringer's solution (*Sacerdote de Lustig, 1942*).

The manner in which the functional activity of the chick embryo's heart can be modified by the action of calcium and potassium was investigated thoroughly by *Murray (1938a)*. His experiments, which cannot be described in detail for lack of space, consisted of transferring excised hearts, mostly from 3-day embryos, from various solutions to others of different composition. In general, transferral involved a change to a solution containing either more calcium or more potassium than the first solution. The hearts themselves were considered as being potassium-rich, if the first solution contained potassium, or potassium-poor, if the first solution lacked potassium or consisted of a mixture of sodium chloride and potassium chloride. The condition of potassium poverty was assumed on the basis of the observation that hearts lose potassium in a solution of sodium chloride, in a mixture of sodium chloride and calcium chloride (though somewhat less rapidly), and probably to some extent in a mixture of sodium chloride and potassium chloride (*Watchorn and Murray, 1938*).

It was observed that an absolute or relative increase in the amount of calcium stimulated the heartbeat in all cases (unless the second solution contained much more potassium than calcium). The stimulation was permanent in potassium-rich hearts but temporary in potassium-poor hearts. In the latter, the initial period of stimulation was succeeded by stoppage, which in turn might be permanent (if the second solution contained no potassium) or temporary (if potassium was present in the second solution).

As in other investigations (*Pickering, 1893a; Lewis, 1929*), it was found that a relative or absolute increase in potassium always led to depression of frequency or to cessation in diastole. The effect required several minutes to appear in potassium-rich hearts but occurred suddenly in potassium-poor hearts. If calcium was present in either the first or second solution, or both, stoppage was followed by a resumption of beating for long or short periods; but if calcium was absent from both solutions, the heartbeat did not return.

Analysis of the observations appeared to indicate that calcium in the medium favors beating, but that its effect depends upon the availability of potassium; also, calcium acts at the surface of the cells, whereas the conjoined action of potassium is exerted from the interior of the cells. Like the stimulation due to calcium, the depressing effect of potassium is a surface phenomenon, and potassium within the cells opposes potassium in the medium. It was concluded, therefore, that the influence of calcium and



potassium depends upon the balance between two ratios, as follows; calcium at the cell surface/potassium within the cell; and potassium at the cell surface/potassium within the cell. Within certain limits, an increase of superficial calcium relative to internal potassium stimulates the heart-beat; if the increase exceeds these limits, or if the ratio is decreased, beating stops. Contraction is also depressed when the ratio of superficial to internal potassium is increased.

### Conduction in the Embryonic Heart

In the adult bird's heart, the conduction of stimuli is accomplished by specialized and ordinary muscle fibers. The exciting impulse originates in the sinoatrial node, which is a small aggregation of specialized muscle tissue close to the valvulae venosae. From here, the stimulus is carried by specialized muscle fibers (Purkinje fibers) to the ordinary auricular musculature, which in turn relays it to the specialized tissue of the auriculoventricular node, located in the interatrial septum. The fibers of the auriculoventricular bundle in the interventricular septum then pass the impulse from the auriculoventricular node to the ventricular musculature, which is activated outward from the endocardium to the epicardium by small branches of the bundle. In its conduction system, therefore, the bird's heart is similar to the mammalian heart. It resembles the reptilian heart also, because it possesses an auriculoventricular ring of Purkinje fibers; but this ring exists only on the right side of the heart (*Davies, 1930a, 1930b*). The specialized conducting pathways in the avian heart are thus more complex than those of the mammalian heart, doubtless because of the relative rapidity of the heart rate in birds.

The conduction system is demonstrable with great difficulty in the adult avian heart, and it is not known when it differentiates. Since the interventricular septum, which contains the auriculoventricular conduction bundle, is not complete before the fifth incubation day, it is obvious that conduction prior to this time (and probably for some time afterward) must be from cell to cell.

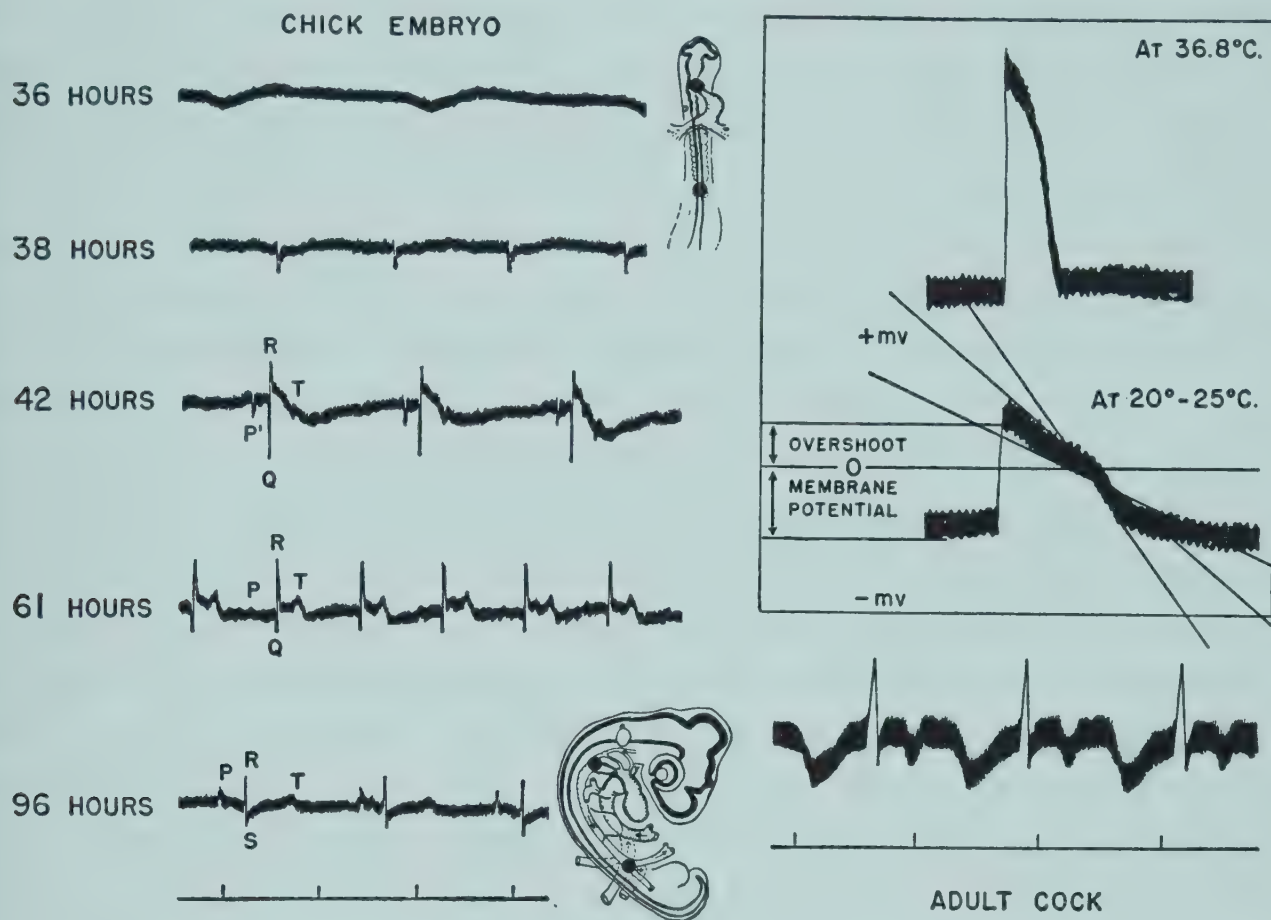
Conduction through the adult heart is evinced by an action potential wave. The passage of this wave is recorded in the electrocardiogram, the deflections of which result from the depolarization and repolarization of the tissue under the electrodes. The avian electrocardiogram exhibits the same components as the mammalian. The *P* wave is associated with auricular systole and the *QRS-T* complex with ventricular systole. The *P-R* interval represents delay of the stimulus at the auriculoventricular node, the *QRS* interval the time of ventricular activation, and the *T* wave the repolarization of the ventricular surface.

During the period when the various primitive regions of the chick embryo's heart are differentiating and beginning to beat, associated changes



occur in the character of the electrocardiogram. Long before the specialized conducting system of the heart comes into existence, the electrocardiogram has assumed practically its definitive configuration.

There is general agreement as to the order in which the electrocardiographic components become recognizable, but the actual time when each wave is first seen has been variously reported, no doubt because of the



**Fig. 295.** Developmental changes in the electrocardiograms of the heart of the chick embryo. (Redrawn with modifications after Bogue, 1933; Hoff, Kramer, DuBois, and Patten, 1939.)

The general changes occurring with development of the embryo are shown schematically at the top right of the plate as determined by Wertheim-Salomonson (1913) from older chicks than those from which the embryonic cardiographs here are reproduced. Note the similarity between the schematic and the actual cardiographs. At the lower right the electrocardiogram of the adult cock is shown for comparison. (Redrawn after Bogue, 1933.)

In the electrocardiograms reproduced, 5 microvolts = 1 mm. The QRS complex appears first. The P wave is inverted when first distinguishable (P' in electrocardiogram of 42-hour chick).

The electrodes were applied at the points indicated by circles in the small drawings at the right of the 36-hour and 96-hour electrocardiograms.

different techniques used by investigators. In the experience of a number of workers, the earliest tracing obtainable is in the form of a slowly undulating, or sinusoidal, curve (Fig. 295). Electrocardiograms of this type have been yielded by embryos of various ages, including 50 to 60 hours (Wertheim-Salomonson, 1913; Cluzet and Sarvonat, 1915), 48 hours (Matsumori, 1930), 36 hours, or 15 somites (Hoff, Kramer, DuBois, and Patten,



1939; Patten, 1939, 1944), and 25 hours, or 9 somites (Olivo, Petralia, and Ricamo, 1946a, 1946b, 1946d; Olivo, 1947). Other observers have failed entirely to record electrocardiograms of sinusoidal configuration (Külbs, 1920; Spadolini and DiGiorgio, 1921; Bogue, 1933).

The emergence of a more complex pattern has also been placed at widely varying ages. According to the earliest report, two sharp crests (or rapid waves) corresponding to the *P* and *R* components appear after 5 or 6 days of incubation; the *T* wave is distinguishable after 8 days; and the *Q* and *S* waves can occasionally be seen at the end of 10 days (Wertheim-Salomonson, 1913). Subsequent investigations have considerably advanced the time of appearance of every electrocardiographic component. The first rapid wave (cf. Fig. 295) has been successively identified at the 50-hour stage (Spadolini and DiGiorgio, 1921), at the 15- to 16-somite (38- to 40-hour) stage (Bogue, 1933; Hoff, Kramer, DuBois, and Patten, 1939), and at stages of 11 to 14 somites (Olivo, Petralia, and Ricamo, 1946a, 1946b, 1946d; Olivo, 1947). This rapid component has been interpreted as an *R* wave, or the initial manifestation of the *QRS* complex (Hoff, Kramer, DuBois, and Patten, 1939). It has been said that the *R* wave begins as an extremely small oscillation preceding the slow sinusoidal wave and later becomes more marked (Olivo, Petralia, and Ricamo, 1946a, 1946b, 1946c, 1946d; Olivo, 1947). The presence of *P*, *R*, and *T* waves (cf. Fig. 295) has been remarked at 60 hours (Bogue, 1933), 50 hours (Külbs, 1920; Spadolini and DiGiorgio, 1921), and 42 hours (Hoff, Kramer, DuBois, and Patten, 1939), that is, at stages of 20 to 30 somites. Lagen and Sampson (1932) made the statement that multiphase tracings are obtainable from 36-hour chick embryos, but provided no illustrations or further description. In general, it has been found that the adult type of electrocardiogram is approximated by the beginning or the end of the third day (Külbs, 1920; Spadolini and DiGiorgio, 1921; Robb, 1929; Matsumori, 1930; Bogue, 1933; Katsunuma and Inada, 1933) or during the fourth day (Hoff, Kramer, DuBois, and Patten, 1939). The *Q* and *S* waves, however, have been said to become more distinct on the twelfth day (Katsunuma and Inada, 1933).

It has been noted that the evolution of the chick embryo's electrocardiogram reflects the sequence in which the various portions of the heart appear and assume the function of initiating contraction (Sands, 1927; Hoff, Kramer, DuBois, and Patten, 1939). The early sinusoidal curve and the first rapid component (or *R* wave) are thus of ventricular origin, since they are seen before the atrium differentiates; the *P* wave, associated with auricular contraction, appears after the atrial primordium has been added to the heart tube.

The analysis of the early electrocardiogram has been carried somewhat further by Olivo, Petralia, and Ricamo (1946b, 1946d, 1949) and Olivo



(1947). According to their interpretation, the sinusoidal curve is associated with purely sarcoplasmic contraction, the ascending phase expressing a slow depolarization of the membrane of the primitive muscle fiber, the descending phase a slow repolarization. The first rapid component, whose appearance corresponds chronologically with that of the earliest myofibrils, is regarded as a manifestation of myofibrillar contraction. Like the QRS complex, of which it is a rudimentary form, it represents depolarization of ventricular muscle fiber membranes, but a depolarization that is much more rapid than that which accompanies sarcoplasmic contraction. With the first weak contractions of the myofibrils, the rapid (myofibrillar) wave and the slow (sarcoplasmic) wave follow each other. Soon, however, as the number of myofibrils increases, there is an increase in the potential of the rapid wave, since the quantitative effect of depolarization is proportional to the myofibrillar content of the fiber. When rapid depolarization reaches its maximum absolute value, sarcoplasmic depolarization can add nothing to the electronegative oscillation. The slow wave, seen at the T wave, then expresses only the phenomenon of repolarization of the membrane. The electrocardiogram, therefore, may be an electric expression resulting not so much from the anatomical structure of the heart as from the summation of small elementary myocardial oscillations (*Olivo and Posteli, 1942-43*). It is interesting to note that the electrocardiographic records of cultured embryonic heart explants resemble those of the heart *in situ* (*Olivo and Posteli, 1942-43; Bonsdorff, 1951*) but revert to the sinusoidal type after the myocardial cells have dedifferentiated (*Olivo, Petralia, and Ricamo, 1946d*). Tracings of variations in the electric potential of single, dedifferentiated myocardial fibers in cultures are in the form of slow oscillations lacking rapid components (*Olivo, Petralia, and Ricamo, 1946c; 1946d; Olivo, 1947*); but the record of the action potential of an individual, pulsating fiber of the 3- to 7-day chick embryo's heart *in vivo*, obtained by the use of a microelectrode, is in the form of a sharply ascending limb representing depolarization and a curved, more slowly descending limb representing repolarization (*Fingl, Woodbury, and Hecht, 1952*). The repolarization limb can be divided into three phases (cf. Fig. 295). The character of the membrane action potential of an individual fiber does not change significantly with age (at least within the first week of incubation), but the rate of repolarization is retarded somewhat by a decrease in temperature.

The electrocardiogram also yields information regarding the rate of stimulus conduction during the period when it must be assumed that conduction is from cell to cell (*Johnstone, 1924*). In electrocardiograms of chick embryos ranging from 50 hours to 5 days in age, the P-R (auriculo-ventricular) interval is 0.09 to 0.15 sec. (*Spadolini and DiGiorgio, 1921; Bogue, 1933*), or but little more than the interval (0.06 sec.) seen in



electrocardiograms of the adult chicken heart. The adult heart, however, is about forty times as long. Apparently a slow conduction rate in the embryonic heart compensates for its small size. Using a photographic method, Fano and Badano (1890b) measured the velocity of transmission of the peristaltic contraction wave in the 3-day chick embryo's heart and found it to be 3.6 to 11.5 mm. per second. Electrocardiograms of 7- to 12-day atria and ventricles separately show that the curve of each is diphasic, consisting of a rapid and a slow component. The action potential wave of the atrium lasts 0.1 to 0.15 sec., that of the ventricle 0.3 to 0.55 sec. (*Katsunuma and Inada, 1933*).

The purely myocardial nature of conduction is shown by the fact that excitation can be transmitted from one cultured embryonic heart fragment to another as soon as a connection is established between them. Explants that have been pulsating asynchronously then develop a synchronized beat. Conduction apparently takes place via protoplasmic "bridges" that form anastomoses between the cells migrating from the different explants (*Fischer, 1924; Olivo, 1925a, 1926a, 1948; Bisceglie, 1929; Olivo and Ricamo, 1948*). Rows of as many as twenty-one fragments of embryonic chick heart have become functionally synchronized (*Olivo and Ricamo, 1948*). Synchronization has been seen to take place between explants from embryos of different incubation ages within the range of 3 to 9 days (*Businco, 1943*) but is achieved with greater ease between explants from younger embryos (*Businco, 1943; Olivo, 1948; Olivo and Ricamo, 1950*); the age of 4 to 5 days is said to be optimum (*Olivo, 1948*). Ventricular fragments become synchronized more readily than atrial (*Olivo and Ricamo, 1950*). Synchronous pulsation has also been established between fragments of the chick embryo's heart and explants from embryonic hearts of the pigeon, *Columba livia* (*Olivo, 1926a*), the mouse, and the guinea pig (*Leone, 1946*).

Electrocardiographic studies of serially synchronized explants (*Olivo, 1948; Olivo and Ricamo, 1950*) have shown that such explants contract not with absolutely perfect synchronization but in measurable succession. The speed of conduction is of the order of centimeters or decimeters per second but varies considerably from one part of a series to another (for example, it was found to range from 7.6 cm. per sec. to 66.7 cm. per sec. in one series). It is slowest across the anastomotic bridges; also, it is slower in atrial than in ventricular fragments.

### *The Effect of Cations on Conduction*

The same cations that are important modifiers of cardiac rate can also affect conduction in the chick embryo's heart. A condition known as microfibrillation, which seems to be a disturbance in conduction, can be produced in the isolated heart of the chick embryo by solutions containing



excessive amounts of calcium, potassium (Olivo, 1924a; Bucciardi and Bisceglie, 1929; Murray, 1935a, 1938a, 1938b), magnesium (Murray, 1935a; Rondinini, 1939), lithium (Murray, 1938b), or barium (Rondinini, 1939). Egg albumen is also capable of providing microfibrillation because of its high content of calcium and potassium (Murray, 1934b). The condition is usually reversible and can be removed by returning the heart to a balanced salt solution.

The descriptions of microfibrillation given by Olivo (1924a) and Murray (1935b) are in essential agreement. The phenomenon appears as a loss of coordination, not between large groups of contracting cells, as in the more familiar macrofibrillation, but between very small groups of cells, or even between single cells; in fact, different parts of the same cell may contract independently. Fibrillation of this type may affect the entire heart or may be confined to large or small areas within it. Fibrillary contractions are of smaller amplitude than coordinated contractions, and their frequency is much higher, though it diminishes from the venous to the arterial end of the heart.

Fibrillation, when produced by excessive potassium, usually does not appear until the normal heartbeat has been suppressed; but when caused by excessive calcium or magnesium, it may occur simultaneously with coordinated beating (Murray, 1935a, 1935b). Normal contractions then may be preceded by a brief trembling caused by fibrillary contractions, or they may alternate with periods of fibrillation (Murray, 1935b). There is also a close relation between the presence of potassium and the induction of fibrillation by calcium, for calcium fibrillation occurs only in the absence of potassium in the medium, or in potassium-poor hearts, and is abolished by solutions containing small quantities of potassium (Olivo, 1924a; Murray, 1938a). When potassium is present in the medium in large amount, however, it produces fibrillation, especially in potassium-poor hearts, and does not oppose calcium fibrillation (Murray, 1938a, 1938b). These observations were interpreted by Murray (1938a, 1938b) as indicating that potassium within the cells opposes fibrillation by either calcium or potassium in the medium, fibrillation therefore being due to an abnormally high increase in either cation at the cell surface relative to intracellular potassium. From this it was argued that fibrillating action is not a specific property of cations but depends on their electrical positivity, and that fibrillation is produced by a change in the potential gradient across the cell membrane, that is, it is related to a high surface positivity.

It was suggested by Murray (1935b) that fibrillation is the visible expression of impaired conduction, which dissociates coordinated beating because new cycles of activity start before the old die away; secondary automatic contractions then set up secondary impulses, with the result that a number of "entangled" impulses exist simultaneously.



A different explanation of fibrillation was given by Olivo (1924a). In his opinion normal beating is the result of the synchronous activity of both sarcoplasm and myofibrils; fibrillation is due to incoordination of myofibrillar contraction. This view was based chiefly upon the observation that fibrillation cannot be induced before the stage of 11 somites, or approximately the time when myofibrils begin to differentiate.

### Excitation by Applied Stimuli

The excitability of cardiac tissue is evinced by an increased rate or altered rhythm when a stimulus is applied. Electricity has been the most commonly used stimulus, but various types of radiation have also been employed.

Adult cardiac muscle responds to electrical excitation in a characteristically rhythmic fashion. Unlike skeletal muscle, it does not exhibit tetanus upon the application of very rapidly repeated electrical stimuli (that is, a tetanizing current), but instead accelerates its rate. This difference is due to the fact that the refractory period of cardiac muscle is very long and also to the fact that the elements of the heart musculature are refractory simultaneously, because of their intimate structural integration. Consequently, when electrical stimuli succeed each other at short intervals, some of the stimuli must of necessity fall within the refractory period. This period coincides with the greater part of systole. Close to the end of systole, the heart becomes "relatively refractory," or subnormally excitable, and gradually recovers its normal excitability from this time until the end of diastole. Thus a single induction shock is ineffective until the end of systole, when it elicits an extra systole of less than normal amplitude, followed by a compensatory pause longer than the usual diastolic pause; but by the end of diastole, stimulation calls for an extra systole of normal amplitude. Nevertheless, the response of the heart is always maximal for its state at the time of excitation.

Electrical stimuli have no effect on the chick embryo's heart prior to the time when contraction begins spontaneously (Johnstone, 1925; Olivo, 1925a). It seems likely that the properties of excitability and autonomous contractility develop simultaneously in the sarcoplasm of the myoblast, for Olivo (1925a) found it possible to elicit a slight acceleration of the heart-beat in response to the application of an induced current at the 8- to 10-somite stage, or as soon as functional activity has started.

Once it has developed, the heart's irritability to electric stimuli increases rapidly (Olivo, 1925a). Johnstone (1925) observed that the acceleration of frequency in response to a tetanizing current was moderate at 60- to 70-hour stages and marked thereafter. Fano (1885), investigating the excitability of the heart in embryos 3 to 12 days old, noted a consistent increase in this property (as indicated by a decrease in the strength of



induced current required to produce acceleration) up to the sixth day, followed by an irregular decrease from the eighth to the tenth day and a further marked increase thereafter. Fano and Badano (1890*b*) reported an early increase in the excitability of both the excised atrium and the excised ventricle.

Fano (1885) pointed out that excitability, by increasing with age, follows a course that is the reverse of the trend exhibited by the property of automaticity. Therefore, Fano and Badano (1890*b*) reasoned that the atrium, possessing a higher degree of automaticity than the ventricle, should be less excitable. In apparent substantiation of this hypothesis, they succeeded in stimulating the excised ventricle electrically at the 37-hour stage but obtained no response from the atrium until the forty-sixth hour. The atrium was found to have a consistently lower excitability than the ventricle until the end of the third day, although it retained its irritability longer after excision. On the other hand, Johnstone (1925), using unipolar excitation, observed that the ventricle (*in situ*), isolated by ligation at the atrioventricular junction, does not become irritable to tetanizing currents or to single induction shocks until the end of the fourth day, or considerably later than the atrium, and that the isolated bulbus develops irritability during the second half of the fifth day.

At all ages, myocardial excitability by electricity is influenced by environmental temperature. As the temperature drops, the threshold of stimulation rises (Fano, 1885).

The degree of acceleration in cardiac frequency is proportional to the strength of induced current applied (Preyer, 1885). This relationship is apparent at the time the heart begins to function (Olivo, 1925*a*) as well as in embryos 2.5 to 6 days old (Pickering, 1896). When the intensity of current is increased beyond a certain level, the heart responds by beating at an extremely rapid rate, which gradually diminishes; diastoles are incomplete, and the heart finally stops in systole (Preyer, 1885; Bottazzi, 1896; Pickering, 1896; Olivo, 1925*a*). This response occurs more frequently in younger embryos (Olivo, 1925*a*). Pickering (1896) noted that currents of sufficient strength to produce "delirium cordis" (macrofibrillation) in embryos less than 6 days old cause cardiac inhibition in older embryos, although the latter still exhibit delirium when the intensity of current is increased still further. He considered the appearance of inhibition to be related to vagal innervation of the heart.

Pickering (1896) also found that strong stimulation of the embryonic heart may be followed by a temporary increase in excitability. After a short application (3 or 4 minutes) of an induced current strong enough to produce either delirium or inhibition (depending upon age), there is a brief period during which these conditions can be provoked by a less intense current. Stimulation for 30 minutes with a weaker current was



found to have the opposite effect; that is, it decreased excitability. Szepsenwol (1947) obtained comparable results with cultured fragments of 2- to 5-day chick hearts. After being stimulated with ten to fifteen condenser discharge shocks of 2 volts each, delivered at the rate of one or two per second, such myocardial fragments not only show no fatigue but become irritable to shocks of one half to three quarters of the initial strength.

According to Olivo (1925a), single induction shocks produce extra systoles as early as the stages of 9 to 11 somites, but the compensatory pause is never seen at this time; instead, stimulation is followed by a period of increased frequency, the duration of which is proportional to the intensity of the stimulus. Johnstone (1925) noted that a pause does not follow acceleration produced by a tetanizing current until the embryo has been incubated at least 70 hours. Bottazzi (1896) remarked that the phenomenon of the compensatory pause occurs in embryos aged 11 days or more.

In studying the effect of induction shocks applied singly or as a series to embryos more than 60 hours old, Pickering (1896) concluded that the effect depends upon the frequency and the strength of the shocks, as well as on the age of the embryo and the environmental temperature. Each shock, if applied in diastole, produces an extra systole; but when the number of shocks per second exceeds a certain frequency which is variable in 2.5- to 5-day embryos and about half the heart frequency in older embryos, the effect is then the same as that of an interrupted (induced) current (that is, either delirium or inhibition). In embryos old enough to exhibit inhibition in response to sufficiently frequent shocks, elevated temperature (42° C.) favors the appearance, instead, of delirium; inhibition again occurs, however, when the strength of the shocks is increased. At a subnormal temperature (28° C.), the reverse is true; that is, inhibition is favored, and an increase in the strength of the shocks produces delirium. Also, Pickering (1896) remarked that a few strong shocks at long intervals produce the same effect as weaker, more rapidly delivered shocks.

Unlike Preyer (1885), Pickering (1896) observed that the chick embryo's heart can be stimulated by a constant current. He found a constant current of 1 milliampere sufficient to accelerate the heartbeat in embryos of 2.5 to 12.5 days' incubation, by an average of six beats per minute. Pickering's statements appear to indicate that the response to constant currents does not change with age. Murray (1934a) remarked that hearts from 2- to 11-day embryos exhibit microfibrillation either during or after the passage of a constant current.

The excitability of the excised heart does not disappear when the heart stops beating, for an adequate electrical stimulus may cause the heart to resume its function for a time (Fano, 1885; Bottazzi, 1896). As in the heart *in situ*, the rhythmic effect produced varies with the strength of the



induced current applied (*Bottazzi, 1896*). Szepsenwol (1947) found that fragments of myocardium that have stopped beating at room temperature contract in response to condenser discharge shocks and that the threshold of stimulation from the second to the fifth incubation day is a shock of 2 or 3 volts for 0.1 to 0.2 milliseconds. Also, Szepsenwol (1947) observed no change in the excitability of such fragments after a week of culture in plasma and embryo juice. Fano (1885) reported that the excitability of hearts held in 0.75 per cent sodium chloride solution diminishes rapidly as the time after excision increases but can be returned to a normal level by renewal of the solution.

As Pickering showed, it is possible to reverse the direction of the cardiac contraction wave by reversing the direction in which a constant current flows through the embryonic heart. Between the ages of 40 and 60 hours, a current of 1 milliampere is sufficient to produce reversal; in embryos 2.5 to 12.5 days old, a current of as much as 15 milliamperes may be required, the heartbeat thus becoming more difficult to reverse by this method as the embryo increases in age. According to Johnstone (1925), a single induction shock applied to the ventricle during a compensatory pause does not reverse the sequence of atrial and ventricular contraction until the ventricle becomes independently excitable by electrical stimuli at the end of the fourth day. Similarly, complete reversal of the contraction wave by stimulation of the bulbus during a pause cannot be achieved until the bulbus develops irritability at the end of the fifth day.

Ultraviolet point radiation of the embryonic chick heart acts as a stimulus when the exposure is short and it is applied to the sinus or the auricle (*Hinrichs and Warrick, 1931*). Long exposures (40 seconds or more) retard the heart rate (*Hinrichs, 1931*); and, after the heartbeat has thus been slowed, radiation of the sinus or the auricle can change the ratio of atrial to ventricular beating from 1:1 to 2:1 or 3:1 (*Hinrichs and Warrick, 1931*).

Exposure to the radiation from 0.005 mg. of radium bromide for 10 to 15 minutes augments the frequency and the intensity of contractions in cultured fragments of the 3- to 9-day chick embryo's heart and causes contractile activity to resume in cultured atrial fragments that have ceased pulsating; it has no revivifying effect, however, on ventricular fragments that have stopped beating, or on fragments that have been paralyzed by the action of potassium chloride. Longer exposure (40 minutes) arrests contractile activity. The same effects are observed upon the addition of a drop of a 0.1 per cent solution of uranyl ( $\text{UO}_2$ ) acetate (in 8.5 per cent sodium chloride) to the culture medium (*Bisceglie and Bucciardi, 1929*). Uranium acetate is a temporary stimulant in concentrations of 0.5 to 1.75 per cent but stops contraction in a concentration of 2 per cent (*Bucciardi and Bisceglie, 1929*).



### Characteristic Responses to Drugs

Many drugs and chemical substances of animal or vegetable origin, including hormones, may affect the activity of the heart, either by accelerating or retarding the rate of contraction, strengthening or weakening the force of the heartbeat, lengthening or shortening some phase of the cardiac cycle, or causing a sudden or a gradual cessation of function. In general, the effect of a drug may be due to its influence on either the muscular tissue, the conductive tissue, and/or the neural apparatus of the heart. Sometimes cardiac metabolism is affected, and often more than one mechanism is involved. In the embryo, a drug may have the same effect as in the adult, or a different effect, depending upon various factors. The amount or concentration of the substance administered, for example, is of great importance in determining the reaction of both embryonic and the adult heart. Environmental temperature may also modify the action of certain drugs during the embryonic period; and the atrial and ventricular portions of the embryonic heart may differ in their susceptibility to the same drug. Another factor is the age of the embryo; thus there can be no question of any neural mediation at a time prior to the innervation of the heart. In the chick, therefore, neural responses are excluded before the latter part of the third incubation day, since fibers of the vagus nerve reach the bulbus at the sixty-eighth hour and the atrium at the ninety-sixth, at the very earliest. Many investigations have shown, nevertheless, that the embryonic heart reacts in a surprisingly mature manner to numerous drugs, especially those which exert a direct effect on the myocardium.

#### *Acetylcholine and Adrenaline*

The action of acetylcholine and adrenaline on the embryonic heart has received considerable attention because these substances, or substances very much like them, are liberated at the postganglionic nerve endings in the adult heart and mediate the neural control of cardiac function. The effect of acetylcholine is similar to that of vagus stimulation, whereas adrenaline accelerates the heart.

The majority of investigators are agreed that the response of the embryonic chicken heart to acetylcholine does not depend upon the presence of nerves but is a characteristic reaction of the myocardium. Although Fujii (1927*a*) found acetylcholine to have no effect on the isolated heart prior to the fourth incubation day, Burnier (1935) later showed that the 48-hour heart, *in situ*, beats at a retarded rate when acetylcholine is applied to the surface of the blastoderm and differs very little from the 5-day heart in its reaction to this substance. Plattner and Hou (1931) obtained the characteristic response to acetylcholine from chick hearts *in situ* at the 72- to 94-hour stages (Fig. 296), when innervation is barely beginning;



and Fingl, Woodbury, and Hecht (1952) were unable to detect any significant change in the reaction of the heart (*in situ*) to acetylcholine from the third to the seventh day, during the period when innervation progresses almost to completion. The latter authors also found that acetylcholine modifies the membrane potentials of individual myocardial fibers in the same way in innervated and non-innervated, hearts, the modification usually consisting of reduction in resting and action potential amplitudes, decrease in action potential duration, and incomplete depolarization. According to Hsu (1933), who observed 2- to 4-day hearts *in situ* and fragments of 1.5- to 20-day hearts *in vitro*, the effect of acetylcholine is very transient, and the heart develops a tolerance to the repeated applica-

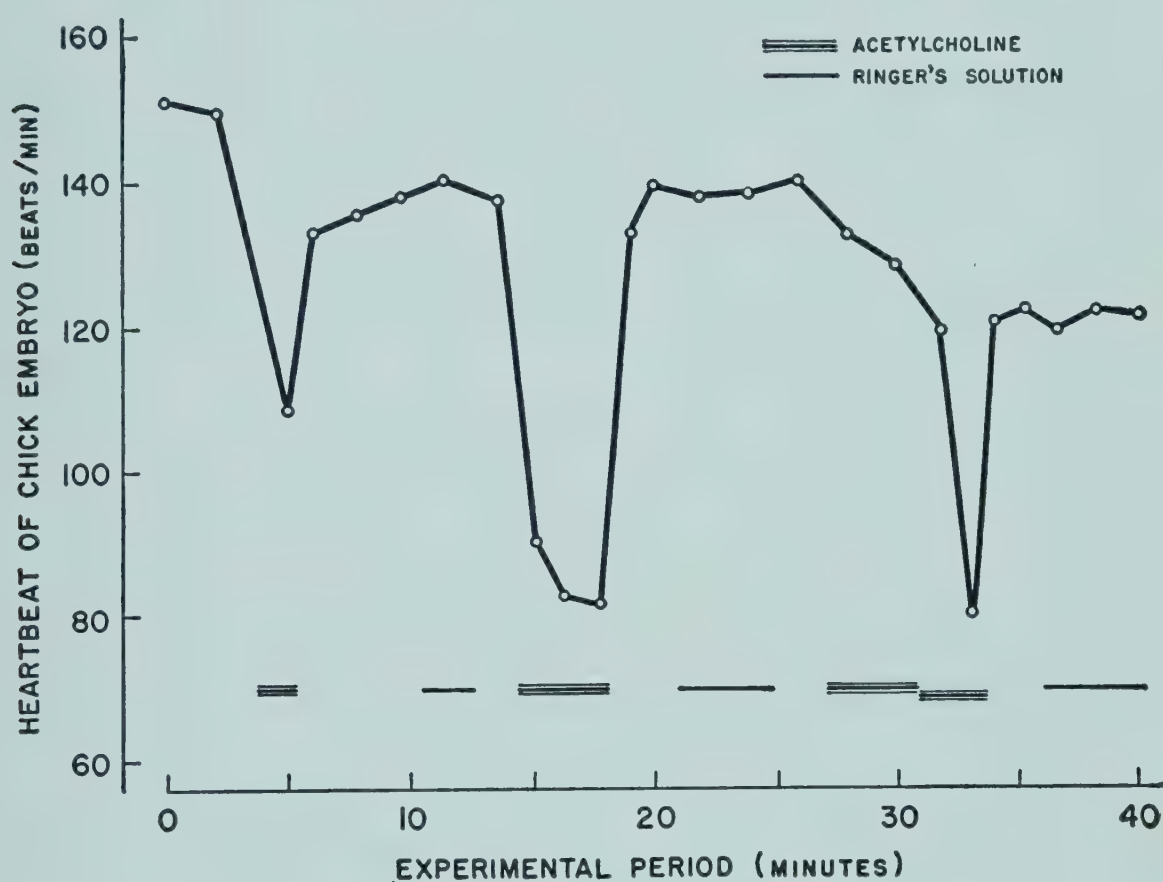


Fig. 296. Effect of alternate applications of acetylcholine, 1:5000, and Ringer's solution on rate of contraction of the heart of the 90-hour chick embryo. (Redrawn after Plattner and Hou, 1931.)

tion of the drug. Furthermore, the great variation in sensitivity to acetylcholine is correlated neither with embryonic age nor with concentration of the drug but with different properties of reactivity inherent in individual embryos. Hsu (1933) stated, however, that the acidity of acetylcholine in too strong a concentration alters the sensitivity of younger hearts and in this manner explained the progressive increase in responsiveness to acetylcholine found by Fujii (1927a) and Markowitz (1931) between the second and the sixth or seventh incubation day. Markowitz (1931) also reported that cardiac explants acquire the ability to react to acetylcholine after they have been cultured for 24 to 48 hours and therefore concluded that the action of acetylcholine depends upon some intermediary substance which



develops during cultivation. Whether or not this conclusion is accepted, it is apparent, at least in the case of the younger explants, that no neural mechanism could have been involved in the appearance of sensitivity in cultures.

The effect of adrenaline, like that of acetylcholine, can be demonstrated long before innervation of the heart begins. Matsumori (1929) and Barry (1950) showed that the excised chick embryonic heart exhibits a definite but transitory increase in frequency at the 29-hour and 40-hour stages, respectively, and Guèlin-Schedrina (1936) observed a temporary cardiac acceleration, followed by retardation, after injecting adrenaline into the 48-hour embryo via the omphalomesenteric vein. These findings differ from those of Fujii (1927*a*), who failed to obtain any pronounced reaction before the end of the fourth day.

During and after the period when innervation of the chick's heart occurs, the effect of adrenaline has been found by several investigators not to change in any consistent way (*Hsu, 1933; Delorenzi, 1938; Fingl, Woodbury, and Hecht, 1952*). On the other hand, Matsumori (1929) stated that the effectiveness of adrenaline attains a maximum at the end of the fourth day and then declines, at least until the sixth day; Markowitz (1931) observed an increase in the sensitivity of the heart to this substance from the second to the sixth day; and Fujii (1927*a*) reported that the heart becomes continually more susceptible to it from the fourth to the tenth day. Experiments with the isolated atrium and ventricle led Matsumori (1929) to conclude that the atrium is accelerated by adrenaline at all times from the third to the seventh day of incubation, whereas the ventricle, on and after the fifth day, is depressed by it. Barry (1950), however, found that all segments of 1.5- to 7-day hearts are stimulated by adrenaline, if the original rate is less than 180 beats per minute, the percentage increase being inversely proportional to the original frequency and particularly marked at frequencies of less than 50 beats per minute.

The specific effect of adrenaline on the membrane potential of the myocardial fiber is shown chiefly by changes in the rate of repolarization, associated with a slight reduction in action potential duration. These modifications remain the same from the third to the seventh day (*Fingl, Woodbury, and Hecht, 1952*).

According to Cornman (1950), fragments of the 9- to 11-day chick embryo's heart that have stopped beating in potassium chloride solution can be returned to activity by the addition of epinephrine to the solution. On the other hand, desoxycorticosterone, desoxycorticosterone acetate, and cortisone acetate do not neutralize the effects of potassium. Desoxycorticosterone at first stimulates and then depresses cardiac pulsations; its depressing effect is more easily achieved in the absence of potassium and can



be removed by serum and sometimes by epinephrine, but never by cortisone. Desoxycorticosterone acetate and cortisone alcohol or acetate do not influence the heartbeat.

It has been reported (*Paes and Pires Soares, 1939*) that the simultaneous addition of adenaline and acetylcholine to cultures of the embryonic chick heart causes nonfunctional hearts to resume their activity. The addition of either substance alone does not produce this effect.

### *Drugs of the Digitalis Group*

The most outstanding effect of digitalin and related drugs on the heartbeat is to increase the force of the contractions while decreasing their frequency. The strengthening effect is due to the direct action of the drug on the onus and contractility of cardiac muscle. The retardation in rate results from inhibition of the sinoauricular node, in turn supposedly due to vagal stimulation by the drug. Digitalin may also produce partial or complete atrioventricular block because it apparently retards or prevents conduction by the atrioventricular bundle.

The digitalis drugs nevertheless exert their influence on the chick embryo's heart at a time not only before vagal innervation has occurred but also before the adult type of conduction system has developed. Thus Kiriara (1931) found that the sensitivity of the chick embryo's heart to digitalein is apparent at the 2-day stage, increases to a maximum within a few days, and then remains unchanged, at least until the tenth day. Das (1947) noted that digitalin in dilutions as high as 1:500,000 is inhibitory to cultured hearts from embryos 2 to 7 days old. The effect of digitalin and strophanthin on the 3-day embryo's heart *in situ* was observed by Pickering (1893a), who reported that moderate doses produce strengthened systoles and weakened diastoles and that large doses rapidly lead to retardation in rate, then to atrial distension and ventricular contraction, and finally to death in ionic contraction. An essentially similar response by the 3-day heart *in situ* was reported by Neter and Witebsky (1935). Occasionally, digitalis drugs cause an initial acceleration of the heartbeat; this phenomenon was seen by Pickering (1893a), Fujii (1927a), Delorenzi (1938), and Paff and Johnson (1938) in chick embryos varying in age from 2 to 5 days.

The characteristic effect of atrioventricular heart block has been observed after immersion of the 48-hour chick embryo's heart in ouabain diluted 1:300,000 (*Paff and Johnson, 1938*). Beginning as occasional dropped ventricular beats, heart block soon increases in severity, until the ratio of atrial to ventricular frequency is 2:1. The ventricle soon steps in diastole, and the atrium ceases to beat after a short time. Sometimes bulboventricular block also occurs. Higher dilutions of ouabain or lowered environmental temperatures delay the appearance of these phenomena. In dilutions of



1:1,200,000, heart block does not take place, and cessation of cardiac activity is preceded merely by a progressive retardation in pulsation frequency. Because of the linear relationship between the dilution of the drug and the time of appearance of atrioventricular block, it has been suggested that the isolated chick embryonic heart may be used in the detection and assay of digitaloid drugs (*Paff, 1940a; DeGraff, Paff, and Lehman, 1941; Lehman and Paff, 1942*).

Since the effect of ouabain is the same when the ventricle is completely isolated from the atrium by transection or ligation, *Paff and Johnson (1938)* concluded that drugs of the digitalis group exert their action by depressing the excitability of the cardiac tissue rather than by interfering with conduction. The observation that hearts or isolated ventricles which have stopped beating under the influence of ouabain, tincture of digitalis, or digitoxin do not respond to electrical stimulation (*Paff, 1940b; Paff and Johnson, 1938*) was cited as evidence supporting this hypothesis. The effects of digitalis on the chick embryo's electrocardiogram have been interpreted, however, as indicating disturbances in conduction. *Lagen and Sampson (1932)* found that the electrocardiogram of the digitalized 35-hour heart shows not only the frequent occurrence of atrioventricular block but also a definite change in the form and direction of the final (slow) phase of ventricular deflection, with directional changes in the T-waves. Furthermore, the membrane action potentials of individual cardiac fibers of 3- to 7-day hearts are also affected in a characteristic way by digitoxin. There is a reduction of action potential amplitude, associated with incomplete depolarization, and an increase in the rate of the first phase of repolarization (cf. Fig. 295). After small doses, there is also so great a decrease in the rate of the second and third phases of repolarization that the duration of the action potential is increased; but after large doses, only the first phase of repolarization appears and action potential duration is much reduced (*Fingl, Woodbury, and Hecht, 1952*).

It has been generally supposed that the poisonous action of drugs of the digitalis group is irreversible and that the drugs cannot be washed out of the heart. Nevertheless, it has been shown that the excised heart (or the ventricle alone) of the 48-hour chick embryo can be arrested by ouabain, digitoxin, or tincture of digitalis and can then completely recover its functional activity in Tyrode's solution (*Paff, 1940b; Paff and Johnson, 1938, 1940*). Beating is resumed more quickly in calcium-free Tyrode's solution or in Tyrode's solution containing sodium hexametaphosphate, sodium citrate, or sodium oxalate, which disionize calcium (*Paff, 1940b*). Of the three drugs, digitoxin is the most difficult to wash out of cardiac tissue and ouabain is the easiest to remove. It is possible to induce stoppage and recovery several times in the same ventricle by placing it alternately in tincture of digitalis and Tyrode's solution. In view of these



findings, it appears that there is no firm chemical union between the drugs and the heart tissue (*Paff and Johnson, 1940*).

### *Other Drugs*

The effects of many other drugs on the heart of the chick embryo have been observed at various times during the embryonic period. For convenience, these drugs are listed below in alphabetical order.

**Alcohol.** The effect of alcohol, applied to the heart of the 75-hour chick embryo, is dependent upon environmental temperature (*Pickering, 1895b*). At 20° C., alcohol (0.01 per cent) produces a temporary slight acceleration of the heartbeat, quickly followed by retardation and stoppage in diastole. Heart action is usually restored when the temperature is elevated. Over the temperature range of 30° to 42° C., the degree of acceleration caused by alcohol increases as the temperature rises, and the depressing effect is slower to make its appearance.

Although a weak solution of alcohol accelerates the embryonic chick heart, a strong solution rapidly brings it to a halt in diastole (*Preyer, 1885*). At the 48-hour stage a 5 per cent solution is a cardiac stimulant, whereas a 10 per cent solution speedily kills the embryo. The lethal effect may be exerted directly upon the cardiac tissue or is possibly an indirect result of congestion of the vessels of the area vasculosa (*Patry and Ferrier, 1934*).

**Ammonia.** In moderate doses, the effect of ammonia applied to the 3-day chick embryo's heart is similar to its direct action on the adult heart; that is, it is a stimulant of cardiac muscle (*Pickering, 1893a, 1895b*). Small doses have no effect (*Pickering, 1893a*), and large doses stop the heart (*Preyer, 1885*) in diastole (*Pickering, 1893a*).

**Amyl nitrite.** Direct application of large doses of amyl nitrite (dissolved in olive oil) to the chick embryo's heart has been seen to result in reduced pulsation frequency, reversal of the contraction sequence, and eventual stoppage in diastole. These effects were interpreted as indicating oxygen deprivation due to the formation of methemoglobin (*Pickering, 1893a*).

**Atropine and muscarine.** Atropine is a cardiac stimulant that neutralizes the action of acetylcholine and therefore antagonizes the inhibition of the heart which is produced by stimulation of the vagus nerve. Muscarine is an antagonist of atropine.

*Pickering (1893a)* found that atropine in large doses either slows the 3-day chick's heartbeat or produces no change whatsoever. According to *Fujii (1927a)*, large doses of atropine have no effect on the chick embryo's heart until the fourth incubation day, after which they are inhibitory. Small doses exert an accelerating influence, but only after the sixth day. *Nordmann and Rüther (1931)* observed that the pulsation frequency of



cultured 4- to 6-day hearts is unchanged by concentrations of atropine that are weak enough to permit survival of the cultures.

Kobert (1886) was unable to demonstrate the effect of muscarine on the heart of the chick embryo of any age but observed its typical depressing action in newly hatched chicks. It appears, however, that this drug, although it has no effect on the 3- and 4-day chick embryo's heart (*Pickering, 1893a*), retards the heart rate after the seventh incubation day and, when given in sufficiently large doses, may then stop the heart in diastole. The effect of muscarine is accentuated at subnormal temperatures, but the stoppage produced at low temperatures can often be removed by the application of heat (*Pickering, 1895b*).

Atropine completely restores the rhythm of 7-day embryonic hearts that have been slowed by muscarine. Hearts that have ceased to beat under the influence of muscarine are returned to functional activity by the action of atropine, but their contraction frequency is reduced (*Pickering, 1895b*).

Since the antagonistic effects of atropine and muscarine are not demonstrable until the embryonic heart has received its nerve supply, it has been concluded that these drugs act through a neural mechanism in the embryonic as in the adult heart (*Pickering, 1895b; Nordmann and Rüther, 1931*).

**Barbiturates.** Sodium phenobarbital has been found to cause cultured fragments of the 2-day chick embryo's heart to pulsate with a progressive diminution of frequency and finally to cease beating entirely (*Acqua and Moravia, 1941a*). This effect is produced in ventricular fragments by a dilution of 1:5000 and in atrial fragments by a dilution of 1:10,000. The depressing action of phenobarbital, although sufficient to overcome the accelerating effect of strychnine, is not in turn counteracted by the latter.

**Caffeine and allied drugs.** Caffeine in small doses accelerates the adult heart by directly increasing the contractility, tonus, and excitability of cardiac tissue; in large doses, the effect is exactly the reverse. Its action on the chick embryo's heart appears to be similar. Fujii (1927b) found that caffeine causes acceleration (of the isolated heart) at all incubation stages from the second to the fourteenth day and that it produces tonic contraction when applied in large doses after the third day. Cultured 3- to 5-day embryonic hearts also respond to caffeine by showing a very transitory rise in pulsation frequency (*Delorenzi, 1938*). The cumulative effect of caffeine, given as a series of doses to the 3-day embryo's heart *in situ*, was demonstrated by Pickering (1893a). According to his account, there is first a slight acceleration of the heartbeat; this is followed by tonic contraction, decreased contraction rate, and cessation in systole. A sufficiently large amount of caffeine given as one dose produces immediate strong tonic contraction and systolic stoppage, without acceleration.

The related drugs, xanthine and theobromine, affect the heart less



markedly (*Pickering, 1893a*). Xanthine causes a primary depression followed by acceleration and an increase in the strength of the beats; but no tonic contraction appears. Theobromine produces acceleration with strong systoles but neither depression nor tonic contraction.

Pickering (*1893a*) suggested that the varying effects of these three drugs may be related to the number of methyl groups in the molecule—xanthine possessing none, theobromine two, and caffeine three. The tonic contraction of cardiac muscle produced by caffeine may be caused, in his opinion, by the introduction of methyl groups into the protein molecule.

Chlorocaffeine, containing three methyl groups and one chlorine atom, produces but slight tonic contraction and elicits much greater acceleration than caffeine. Pickering (*1895a*) explained the difference in the effects of the two compounds as possibly being due to the modification of the physiological action of the methyl groups by the chlorine atom, which tends to produce an atomic condition. The same author remarked that cyanocaffeine affects the chick embryo's heart in much the same manner as hydrocyanic acid, the cyanogen radical thus also overpowering the methyl groups physiologically.

**Chloroform and ether.** Chloroform is said to reinforce the vagal inhibitory mechanism in the adult heart; ether produces acceleration of the cardiac rate.

Pickering (*1893a*) compared the action of chloroform and ether on the heart of the 75- to 80-hour chick embryo. Chloroform was found to slow the heartbeat and eventually to stop the heart in an expanded condition. The ventricle continued to contract after the atrium had succumbed, and there was a reversal of rhythm before activity finally ceased. Ether, on the contrary, acted as a stimulus in moderate dosage and caused a nonpulsating heart to resume its function. In very large dosage it stopped the heart in diastole. Nordmann and Rütger (*1931*) reported that ether (vapor) either caused a temporary acceleration of pulsation in cultured 4- to 6-day hearts or killed the cultures.

**Hormones.** Insulin, injected into the 2-day chick embryo via the omphalomesenteric vein (*Guèlin-Schedrina, 1936*) or applied directly to cultured fragments of the atrium from the 4-day embryo (*Bisceglie, 1929*), may cause a slight initial acceleration of the heartbeat followed by retardation and eventual stoppage; hemorrhage may occur at the 2-day stage. Myocardial fragments that have become anastomosed and are beating synchronously lose their common rhythm after the application of insulin and again pulsate asynchronously, apparently because insulin inhibits conduction by the cells anastomosing the two fragments (*Bisceglie, 1929*).

Thyroxine, injected into the omphalomesenteric vein of the 48-hour blastoderm, causes cardiac acceleration and arrhythmia followed by retardation and a short cessation in diastole (*Guèlin-Schedrina, 1936*). Similarly,



thyroid extract (aqueous) has been found to increase the force and usually the frequency of the pulsations in cultured atrial fragments from the 4-day chick embryo's heart (*Bisceglie, 1929*).

According to *Bisceglie (1929)*, the application of liquor folliculi to cultured atrial fragments from the 5- and 6-day chick embryo's heart causes a more or less rapid diminution in pulsation frequency with a decrease in the force of the contractions.

**Hydrocyanic acid.** When applied directly to the 3-day chick embryo's heart, hydrocyanic acid has the primary effect of decreasing cardiac frequency while increasing the force of systole. After repeated applications of the acid, systoles become weak and the sequence of contraction is reversed. Atrioventricular heart block then occurs, and the heart finally stops in extreme diastole. For a limited time, mechanical stimulation of either the atrium or the ventricle may produce a few normally directed contractions. *Pickering (1893a)*, who observed these effects, suggested that retardation in rate and reversed rhythm arise from interference with normal oxidative processes by the action of cyanhemoglobin. The condition of expansion he attributed to the generic action of hydrocyanic acid as an acid, since dilute lactic and acetic acids also produce expansion. Apparently, the specific action as an acid eventually overcomes the action of the cyanogen radical, since reversal of rhythm no longer occurs upon mechanical stimulation in the final stages of hydrocyanic poisoning.

**Morphine.** Morphine may produce transitory acceleration in the adult heart, but its characteristic effect is inhibitory. It apparently enhances the vagal mechanism, since it causes cardiac acceleration after the vagus is cut.

According to *Pickering (1893a)*, morphine acetate acts as a simple depressant of the 3-day chick embryo's heart when the environmental temperature is subnormal ( $31^{\circ}$  C.). At higher temperatures ( $40^{\circ}$  C.), its retarding effect is complicated by such phenomena as reversal of contraction sequence and alternating periods of rest and rapid beating. The heart finally stops in diastole regardless of the temperature.

The reversed rhythm produced by morphine was explained by *Pickering* as being due to an oxidation inhibiting effect of the drug. He suggested that a decrease in the oxidative capacity of the atrium (which has a higher metabolic rate than the ventricle) may reduce its automaticity to less than that of the ventricle. Reversed rhythm then results because excitation originating in the ventricle starts a contraction wave there.

**Nicotine.** The effect of nicotine on the adult heart is initial retardation followed either by stoppage or by acceleration due to blocking of the vagal action. Large doses also cause acceleration.

*Preyer (1885)* stated that nicotine quickly paralyzes the embryonic heart. According to *Pickering (1893b)*, however, small doses of nicotine cause a moderate acceleration of the heartbeat in the 80-hour chick embryo.



although large doses depress the heart and stop it in diastole. Presumably, these effects represent the direct reaction of the cardiac myoplasm. Fujii (1927*b*) obtained similar responses from the isolated hearts of 2- to 14-day chick embryos but failed to observe the stimulating action of small doses of nicotine until after the fifth incubation day.

**Physostigmine.** This drug enhances the effect of acetylcholine or vagal stimulation. Fujii (1927*b*) found that it inhibits the isolated heart of the 2- to 14-day chick embryo and that it is not antagonized by atropine. After the fifth day, small doses have an accelerating effect.

**Pilocarpine.** The adult heart is first retarded and then accelerated by pilocarpine as the vagus is stimulated and then depressed. The injection of pilocarpine (amount unspecified) into the 48-hour chick embryo slows the activity of the heart; no neural mechanism, of course, is involved in this response (Guèlin-Schedrina, 1936). Pickering (1893*a*) found that the effect of pilocarpine at the 60-hour stage depends upon the dosage administered; small doses accelerate the heartbeat, and large doses quickly depress the heart and stop it in diastole. Essentially the same results were obtained by Fujii (1927*b*), who made observations of 2- to 14-day hearts. Pickering (1893*a*) observed that atropine does not exhibit its characteristic antagonism to pilocarpine in the 60-hour heart.

**Quinine.** Preyer (1885) commented upon the paralyzing effect of quinine on the embryonic heart, and Fujii (1927*b*) reported that quinine exerts an exclusively inhibiting influence on the chick embryo's heart at all stages from 2 to 14 days. Thus the effect of quinine on the embryonic heart appears to be the same as its direct action (in large doses) on adult myocardial tissue.

**Strychnine.** Pickering (1893*a*) found that 0.017 mg. of strychnine depressed the frequency of a 70-hour chick embryo's heart. However, the application of 0.02 mg. of strychnine temporarily accelerated the beating of an 80-hour heart, and a second dose of 0.02 mg. reduced the heart rate until cessation occurred in diastole. Injection of strychnine into the body of the 48-hour embryo produces much the same effects, but injection into the omphalomesenteric vein stops the heart abruptly (Guèlin-Schedrina, 1936).

The effect of strychnine on fragments of 2-day chick embryonic hearts cultured for 48 hours in plasma, Ringer's solution, and embryonic extract was investigated by Acqua and Moravia (1941*b*). In general, the addition of 1 cc. of strychnine (in dilutions of 1:2000 to 1:100,000) to the culture medium produced acceleration. This was followed by arrhythmia and cessation of activity when strychnine in the stronger concentrations was used. The higher dilutions of strychnine stimulated atrial fragments but were without effect on ventricular fragments. The acceleration produced in ventricular fragments by a 1:10,000 dilution of strychnine was in inverse



relationship to the original frequency; thus fragments beating at the rate of thirty-five to forty-five pulsations per minute showed a threefold increase in frequency, but those beating ten to twenty times per minute were accelerated more than sevenfold. The effect of strychnine could be overcome by the addition of 1 cc. of Ringer's solution to the culture, and it appears, therefore, that there is no stable fixation of strychnine by the cardiac muscle cell.

The paralysis produced by high concentrations of strychnine cannot be reversed by acetylcholine and physostigmine, but the effects of the latter substances are neutralized by strychnine (*Sacerdote de Lustig, 1943*). The action of strychnine thus seems to be both toxic and anti-acetylcholinic.

**Veratrine.** Veratrine is a depressant of the adult heart, acting through the vagus center. Fujii (1927*b*) found that in large doses it has a paralyzing effect on the chick embryo's heart after the third day, whereas in small doses it accelerates the heart at any time from the second to the fourteenth day. The depression caused by larger doses takes the form of a gradual lengthening of systole, while diastole is complete; the heart stops in diastole (*Pickering, 1893a*).

According to Pickering (1895*b*), the effect of veratrine on the 75-hour chick's heart depends to a large extent upon environmental temperature. A dose that retards cardiac frequency at 20° C. causes progressive acceleration as the temperature rises from 30° to 42° C.; and a dose that is fatal at 20° C. merely depresses the heart at 30° C. and causes continually less retardation as the temperature increases to 42° C. In other words, heat counteracts the effect of this drug in the chick embryo.

Potassium chloride antagonizes the effect of veratrine, but the reverse does not hold (*Pickering, 1893b*). On the other hand, veratrine can neutralize the effects of acetylcholine and physostigmine, but these substances cannot remove the paralysis produced by veratrine in high concentrations (*Sacerdote de Lustig, 1943*).



## CHAPTER TEN

# *The Urogenital System*

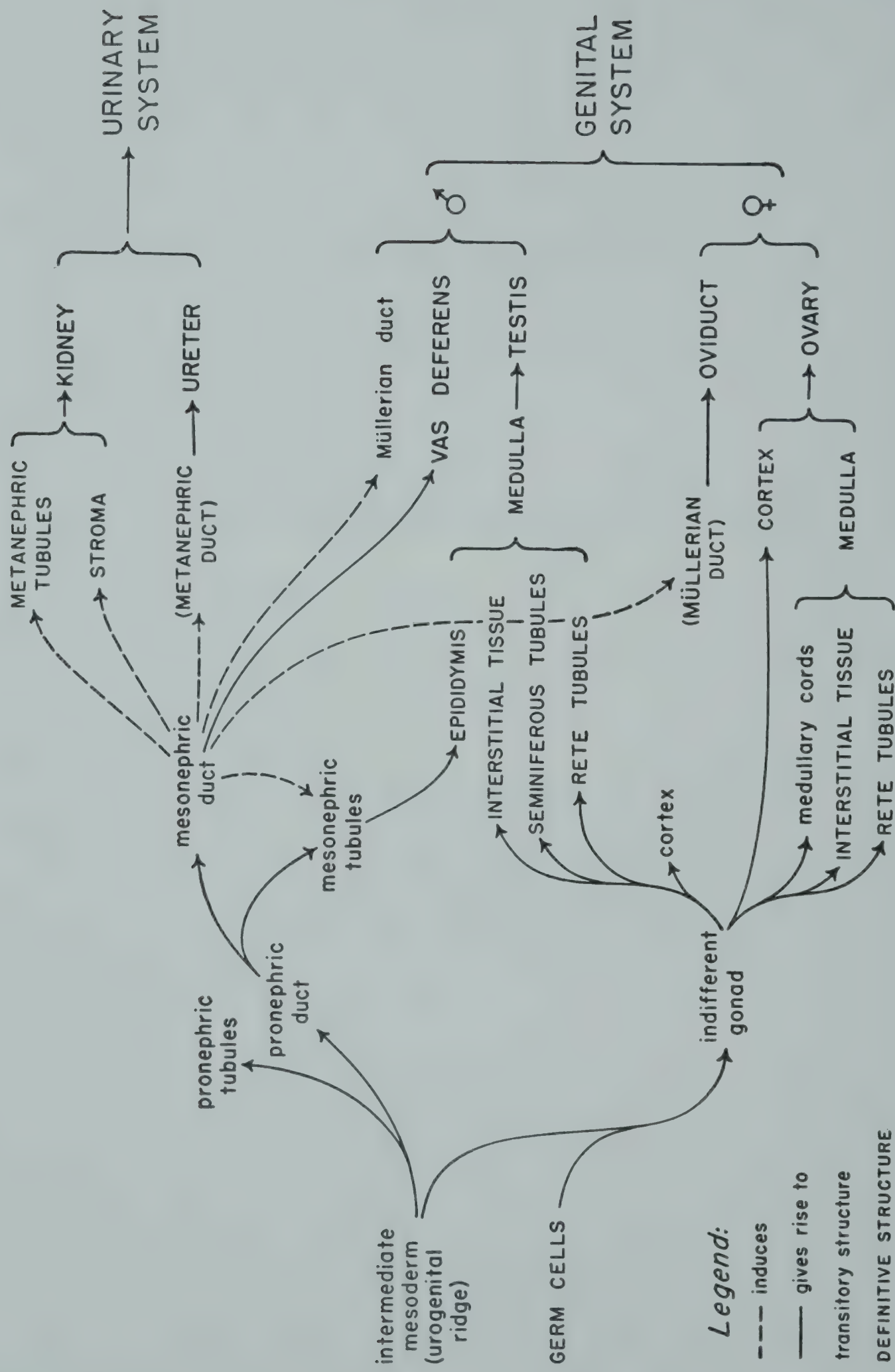
*This system has a dual function,*

*Both of a common origin . . .*

*They serve each other in conjunction:*

*Excrete . . . , and reproduce within.*







# THE UROGENITAL SYSTEM

The excretory and reproductive systems are collectively referred to as the urogenital system because of their close interrelation, both during early development and in the adult. Their location is dorsal to the other visceral organs; in fact, they develop retroperitoneally and project into the coelom later in embryonic development.

The excretory system arises early in embryonic life from the intermediate mesoderm which is situated between the somites and the lateral plate. Kidney development proceeds in three stages. The first nephric structure (pronephros) degenerates promptly, the second (mesonephros) contributes to the formation of the genital system, and only the third (metanephros) remains as the definitive kidney. The chief function of the kidney is the removal of waste products. This is accomplished by a highly specialized blood filtration apparatus, the filtrate leaving the body by a series of ducts.

The gonads appear soon after kidney development has reached its intermediate stage, and are derived from the urogenital ridge. This peritoneal thickening is on the median surface of the mesonephros. After passing through an indifferent stage, sexual differentiation into testes and ovaries occurs. The function of the genital organs is to provide a site for the maturation of the germ cells. These sex cells, however, have a separate origin and a longer developmental history (see Chapter 1). The ducts which convey the excretory and sex products are closely related to the kidneys and gonads in origin. The male genital duct is derived directly from the mesonephric duct, while the oviduct of the female has its origin in the epithelium covering the mesonephros. In the female the mesonephric duct degenerates; in the male, the oviduct degenerates. It should be borne in mind that although the singular noun is often used to designate a paired structure, the plural is implied.

## THE EXCRETORY SYSTEM

In the avian embryo there is a succession of three distinct excretory organs. The pronephros is the first to arise and is the most anterior kidney. It is a nonfunctional vestige suggestive in structure of the most primitive vertebrate kidney. The mesonephros, or intermediate kidney, appears caudal to and soon after the pronephros. It is functional during embryonic life,



excreting ammonia and urea, but not uric acid. It is the permanent excretory organ of *Elasmobranchi* and *Amphibia*. In many adult fishes it serves exclusively as an excretory organ, but in higher forms it becomes associated with the genital system, particularly in the male. In birds, the mesonephros is superseded by the metanephros, which is the functional kidney of adult *Amniota*. The metanephros represents the highest stage of kidney development and is particularly adapted for water reabsorption (loop of Henle) and blood filtration (renal corpuscle). The avian and mammalian metanephroi are morphologically and physiologically quite similar. Mammals however, possess a better developed loop of Henle, a glomerulus used for filtration-reabsorption, and certain histological differences. Grossly, the mammalian kidney is a paired, bean-shaped body while the avian kidney is lobulated and somewhat elongate and of a soft consistency. The avian kidney is composed of a large number of uriniferous or renal tubules which remove urine from the blood. The glomeruli are the capillary tufts which deliver blood to the tubules. Each renal tubule, or nephron, drains into larger collecting ducts until finally urine is carried from the kidney to the cloaca by the straight and narrow ureter.

### The Pronephros or the Primitive Kidney

The pronephros, the most anterior excretory organ and the first to appear, is believed to develop by self-differentiation, growing independently of the surrounding mesoderm and requiring no inducing agent to evoke its development. Besides being vestigial and functionless, it is of short duration and disappears almost completely by the fourth day of chick incubation. In general, the pronephros consists of a series of tubules which open into a common passage, the pronephric duct. The latter degenerates anteriorly and continues posteriorly as the Wolffian duct or mesonephric duct; which in turn serves as the duct for the metanephros. Associated with the pronephric tubules are the glomeruli, small cellular outgrowths into the peritoneum. These open into the coelom by nephrostomes.

**Early determination.** The pronephros in birds has its origin in the nephrotomal mass, which is the mesodermal tissue situated between the somatic and lateral plate mesoderm (Fig. 297). Pronephric tubules develop from the intermediate cell mass at the embryonic stage of very early neural plate formation in the chick (*Waddington, 1938*). At this time somatic, intermediate, and lateral plate mesoderm have been determined. *Hoadley (1926d)* showed that the organization of the pronephros is achieved before the formation of the head-process. Portions of early chick blastoderm, taken even before the localization or differentiation of pronephric primordia, were grown as chorioallantoic grafts. A tissue graft taken from a blastoderm at a transverse level through the broad part of the primitive streak, developed nephric tubules, identifiable as secreting tubules by



the lining of columnar epithelium. A transverse portion from the middle level of a 6-hour blastoderm grew secretory tubules and glomeruli which were intimately associated with some of the tubules. A section from the anterior third of the primitive streak of a 10-hour embryo grew secreting tubules, collecting tubules lined with cuboidal epithelium, and rather typical mesonephric glomeruli.

**Self-differentiation.** The self-differentiating capacity of the pronephros can be demonstrated by making appropriate longitudinal cuts in embryos

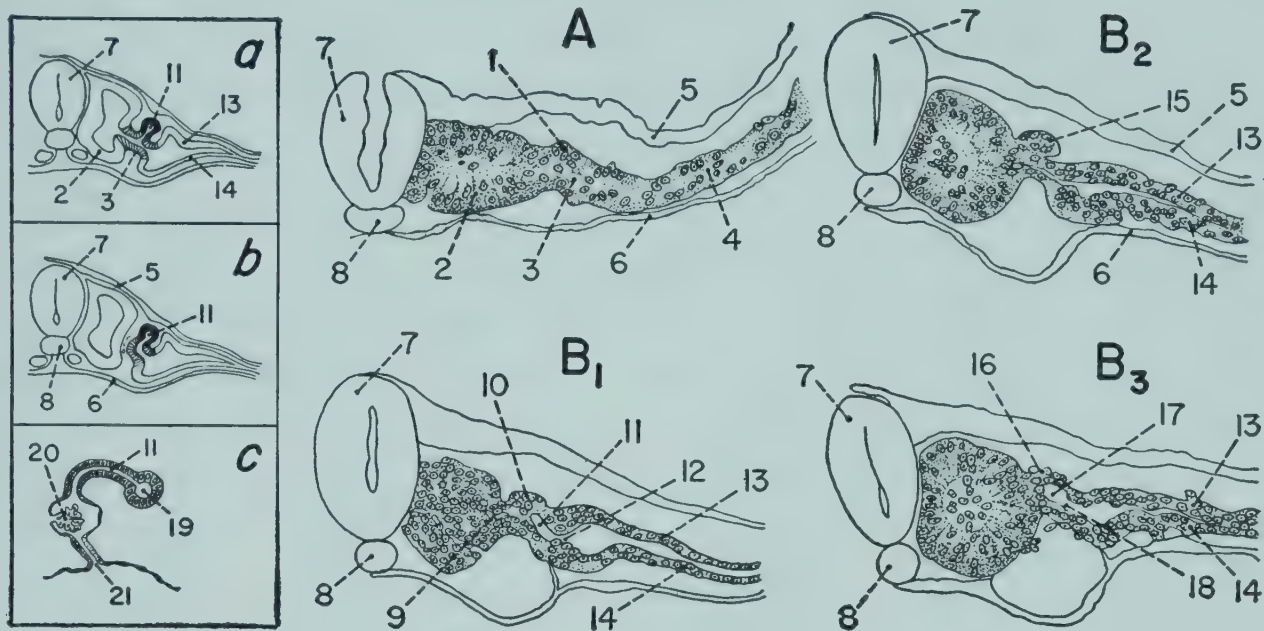


Fig. 297. The development of the pronephric tubule in the chick embryo shown by cross sections. (Redrawn with modifications after Felix, 1906.)

A, primordium of tubule appearing as a ridge in the intermediate mesoderm, 8-somite stage; B<sub>1</sub>, section through middle portion of first tubule of 19-somite embryo; B<sub>2</sub>, section through solid posterior end of first tubule, 19-somite stage; B<sub>3</sub>, section through anterior end of second tubule, 19-somite stage. All  $\times 100$ .

*Insert:* a, diagrammatic representations of tubule formation from dorsal edge of intermediate cell mass; b, subsequent separation from somitic mesoderm; c, completed pronephric tubule.

1, pronephric tubule primordium; 2, somitic mesoderm; 3, intermediate mesoderm; 4, lateral plate mesoderm; 5, ectoderm; 6, endoderm; 7, neural tube; 8, notochord; 9, center of somitic stalk; 10, middle region of first pronephric tubule; 11, lumen of first tubule; 12, nephrostome of collecting tubule; 13, somatopleure; 14, splanchnopleure; 15, solid end of first pronephric tubule; 16, posterior tip of first tubule; 17, lumen of second pronephric tubule; 18, collecting tubule of second pronephric tubule; 19, collecting (pronephric) duct; 20, glomerulus; 21, nephrostome.

of an early neural plate stage (Waddington, 1938). Pronephric tubules may develop independently in the absence of lateral plate mesoderm or somatic mesoderm. The development of pronephric tubules proceeds independently of the inducing effect of Wolffian ducts (Kum  , 1941).

**Tubule formation.** The primordia of the pronephric tubules first appear as paired thickenings of the somatic layer of the intermediate mesodermal mass (Fig. 297-A). In the 10-somite embryo, the paired, solid buds of cells grow in a dorsolateral direction toward the ectoderm from the



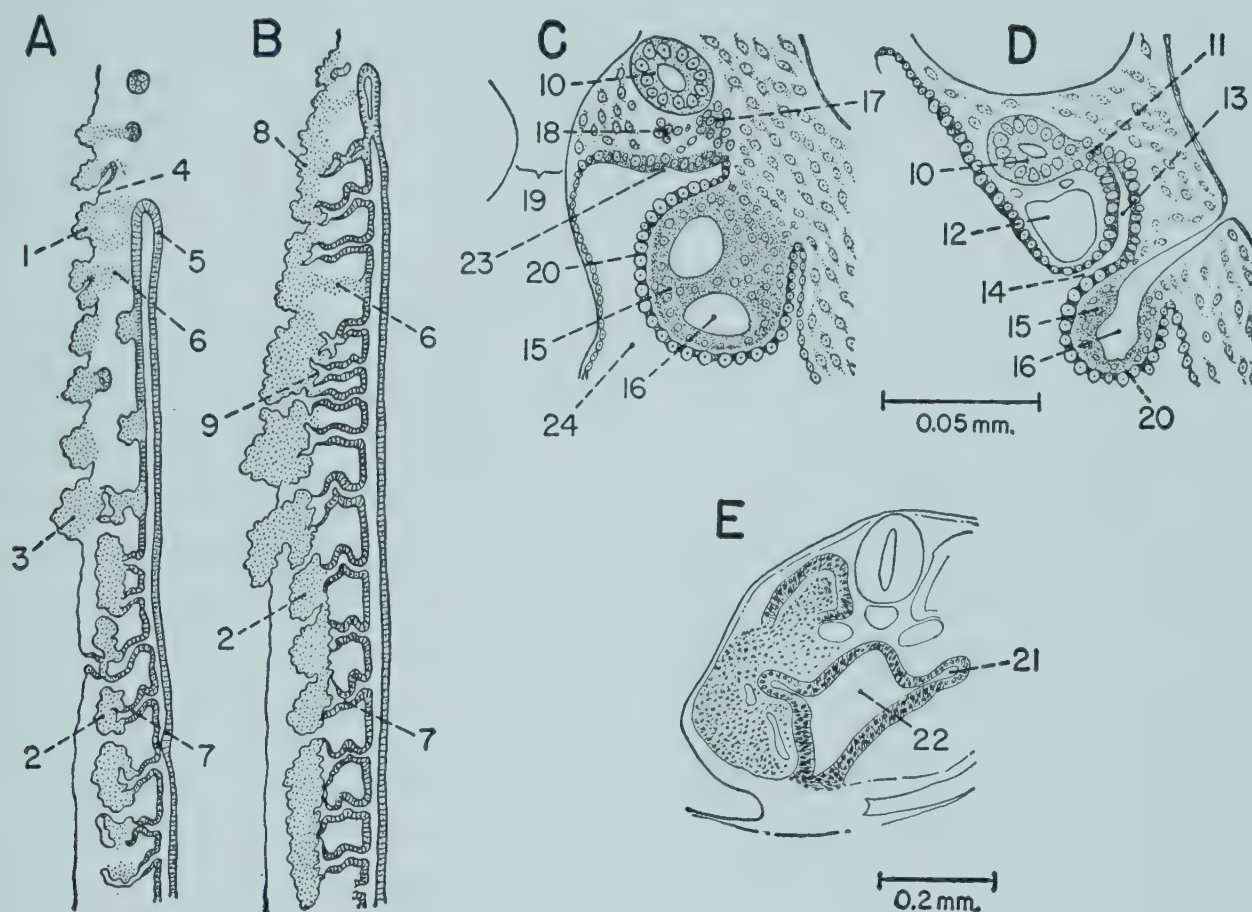
nephrotomal region, which extends from the level of the fifth or sixth somite to the level of the tenth. At 14 somites, the tubules of somites 8 to 15 fuse to form the primordium of the pronephric duct (*Abdel-Malek, 1950*). As the somites continue to appear, the number of pronephric tubules increases until, in the 24-somite chick, there are 11 pairs of tubules extending from the fifth to the sixteenth somite. Development may proceed at different rates on the right and left sides. The nephrotomal mass loses its connection with the somites at the 13-somite stage (Fig. 297—Insert *b*) by becoming converted to mesenchyme medial to the tubules. The portion of the nephrotome between the primary tubule and the lateral plate is maintained and contributes to the supplementary part of the tubule.

**Pronephric duct development.** Soon after the initial growth dorsad, the distal portion of each tubule grows posteriorly and fuses with the tubule of the next segment. The primary cord of cells thus formed is known as the pronephric duct. A little later, it is continued posteriorly as the mesonephric duct. Although the general direction of tubule and pronephric duct development is anteroposterior, Kumé (1941) indicated that cord formation in the 10-somite chick may start at the tubules of somite 10 and proceed anteriorly to segment 7, and is then followed by the typical posterior elongation. He described the tubule-cord development as a process of independent posterior elongation of the cord, while the tubules grow upward and become secondarily attached to the cord.

**Glomerulus formation.** In addition to the primary tubular portion of the pronephros, there is a supplementary part consisting of the coelomic glomeruli. These glomeruli first appear after the pronephros has begun to degenerate and the mesonephros is forming. The initial appearance of glomeruli has been observed in the 30-somite (*Sedgwick, 1881*) and 32-somite chick embryo (*Abdel-Malek, 1950*); during the third day in chicks and during the fourth day in ducks, *Anas platyrhynchos* (*Mihalkovics, 1885*); and in the 64-hour duck embryo (*Davies, 1950*). At their first appearance, the glomeruli of the duck pronephros are large, compact, nephrogenic masses closely connected to the coelomic epithelium and protruding into the dorsal wall of the body cavity (Fig. 298-A). In 12 hours, a rudimentary nephrostome may develop as a slitlike extension of the body cavity into the cell mass. Blood vessels appear within this tissue (Fig. 298-D) before connection with the aorta is established. At 96 hours, eight primordia of external glomeruli have appeared in the duck and these are occasionally connected with the Wolffian duct (*Davies, 1950*). New glomeruli develop between the original ones, thus forming a continuous mass. Internal glomeruli arise somewhat later from the same parent tissue and maintain an intimate relation with the external glomeruli (Fig. 298-B). A glomerulus is considered to be external when it hangs freely into the peritoneal cavity. It is internal when it lies within the Wolffian ridge enclosed within a capsule. An



internal glomerulus with its capsule constitutes a Malpighian body. Both internal and external glomeruli are supplied by the same afferent arteriole. When fusion occurs between their vascular cores, the distinction between external and internal glomeruli is lost. Thus there is an anterior pronephric region consisting of external glomeruli, nephrostomes, and tubules, which is rudimentary in both the duck (*Anas platyrhynchos*) and the chick. An intermediate zone comprises the area in which external and internal



**Fig. 298.** The development and relationship of internal and external glomeruli in the avian embryo. (Redrawn with modifications A and B, after Davies, 1950; C, D, E, after Abdel-Malek, 1950.)

A, longitudinal section showing primordia of eight external glomeruli, and early stages of internal glomerulus showing a fusion zone limited to one set of internal and external glomeruli in the 96-hour duck (*Anas platyrhynchos*) embryo; B, fusion of external glomeruli into a continuous vascular mass, at least eight well-differentiated internal glomeruli, in the 4.5 day duck embryo; C, transverse section through external glomerulus, pronephric duct, and surrounding tissue, in the 72-hour, 35-somite chick (*Gallus gallus*) embryo (in scale); D, external glomerulus with vascular supply, opening of mesonephric tubule into nephrostome in the same (in scale); E, section through cloacal region showing two Wolffian ducts opening into cloaca in the same (in scale).

1, primordium of external glomerulus; 2, internal glomerulus; 3, fusion between external and internal glomerulus representing transition stage from former to latter; 4, rudimentary nephrostome; 5, dilated upper end of mesonephric duct; 6, cord of cells connecting duct to thickened coelomic epithelium; 7, mesonephric tubule passageway between glomerulus and duct; 8, fusion of external glomeruli to form mass of vascular tissue; 9, internal glomerulus with Bowman's capsule; 10, pronephric duct; 11, mesonephric tubule; 12, subcardinal vein; 13, mesonephric tubule lumen; 14, mesonephrostome; 15, mesenchymal mass of external glomerulus; 16, vascular supply to glomerulus; 17, remnants of mesonephric tubule; 18, pronephric tubule remnants; 19, posterior cardinal vein; 20, peritoneal epithelium; 21, Wolffian duct; 22, cloaca; 23, pronephrostome; 24, coelom.



glomeruli are both located. This area is rudimentary in the chick but is extensive and probably functional in the duck (*Davies, 1950*). A caudal zone belonging to the mesonephros contains no external glomeruli.

The number of external glomerular primordia varies greatly. In the chick Sedgwick (1881) observed seven distributed irregularly from segments 11 to 14; Mihalkovics (1885) found that there are five to six primordia in chickens and ducks (*Anas platyrhynchos*), ranging from the eighth to the twelfth somite. Chickens have four to five external glomeruli, according to Renson (1883), and three have been observed in pigeons, *Columba livia*, chickens, and European quail, *Coturnix coturnix* (*Janošik, 1885*). It has been suggested that this inconstant number is the result of a great variability of form and the tendency of the glomerular cell clusters to fuse with one another.

The supply of blood vessels to the glomeruli corresponds to the segmental arrangement of the aortic branches. The aorta is considerably widened over the distance extending from the first well-defined pronephric tubule to the last. This dilation appears at the time the pronephros develops (*Felix, 1906*) and is therefore felt to bear close relation to the early kidney.

The development of the pronephric glomeruli is generally coincident with the appearance of the mesonephric tubules. A certain gradation appears between the posterior glomeruli and the anterior Malpighian bodies of the mesonephros. In the intergrading area, the glomeruli may fuse with the Malpighian bodies to form transitional glomeruli (*Felix, 1906*).

**Degeneration of the pronephros.** By the time the glomeruli are forming, pronephric degeneration has begun. The anterior tubules lose connection with the duct, and both structures are resorbed. In the 21-somite chick there is practically no evidence of the pronephros anterior to the eleventh somite. Between the eleventh and fifteenth somites the pronephric tubules are typically developed and connected with the Wolffian duct. The 35-somite chick embryo may have epithelial remnants of the pronephric tubules and duct as far anterior as the eleventh somite, but from segments 7 to 11 these have atrophied completely (*Sedgwick, 1881*). Nephrostomes may be retained somewhat longer. These appear in the pronephric region of older embryos as funnel-shaped isolated structures composed of coelomic epithelium, disappearing entirely by the sixth day.

The pronephric glomeruli begin to atrophy on the seventh day and are no longer found in the 8- to 9-day chick embryo (*Mihalkovics, 1885*). Glomerular degeneration begins in the chick during the fourth day of incubation and is pronounced on the fifth day (*Abdel-Malek, 1950*). Glomerular breakdown follows a stage when the walls of the cavities in the glomerular tissue become attenuated and break down. The texture of the glomeruli is loosened, and eventual disappearance is the result of progressive fibrosis and shrinkage (*Davies, 1950*).



### The Wolffian Duct

The anterior part of the Wolffian duct may be thought of as the pronephric duct, but in its continued posterior growth it becomes associated with mesonephric structures. It continues to grow caudad by self-differentiation without the addition of adjacent mesoderm, eventually opening into the cloaca. In addition to serving as the excretory duct for tubules developed along its length, the Wolffian duct serves as an inducing agent, evoking the normal development of the mesonephric tubules and Müllerian duct.

#### *Duct Formation*

The appearance of the Wolffian duct is intimately linked with the first mesonephric tubules. It comes into being as the tubules are formed. The duct arises as a solid strand of barely differentiated cells and later acquires a lumen. By this time the duct reaches and opens into the cloaca.

***Time of determination.*** The organization of chick blastoderm material is such that the primordium of the Wolffian ducts appears to be determined in the embryo after 10 hours of incubation. Chorioallantoic grafts of the tissue containing the anterior one third of the primitive streak developed Wolffian ducts which were shorter than normal but were of cuboidal epithelium and not convoluted. In this tissue culture, the two ducts were distinct and did not approach each other or fuse at any point (*Hoadley, 1926d*).

***Early growth.*** The Wolffian duct caudal to the pronephric region, arises by splitting off from the intermediate cell mass as a solid cord of cells (*Gasser, 1877a; Fürbringer, 1878; Sedgwick, 1881; Mihalkovics, 1885*). It has been seen to appear initially in the 8-somite chick embryo as bilaterally paired thickenings of mesoderm in the region from the fifth to the eighth somite (*Gasser, 1877a*) and also as a slight projection in the region from the seventh to the eighth protovertebrae (*Sedgwick, 1881*). According to the account given by Mihalkovics (1885), the cells of the intermediate mass at the level of the chick embryo's fifth segment multiply rapidly in the middle of the second day, and by transverse division, form a small protuberance. This protuberance is the beginning of the ridge of cells representing the early Wolffian duct. Waldeyer (1870, *p. 108*) stated that the anlage in the chick is a solid cord of cells lying dorsal to the intermediate cell mass.

The Wolffian duct grows posteriorly as the somites increase in number. At the 11-somite stage, there are projections from the seventh, eighth, ninth, and tenth protovertebral levels. From the eleventh somite, the rudiment of the duct grows posteriorly as a cord of cells not connected to the peritoneal epithelium (*Sedgwick, 1881*). When the solid cord of cells



has extended from the fifth to the eighth segment, a fine groove appears ventrally in the posterior end of the cord, separating it from the underlying mesoderm except at its anterior end. The anterior attached part is triangular in cross section and is 45 microns high and 32 microns wide; the caudal end is spindle-shaped, 10 microns high and 64 microns wide. The cord consists of undifferentiated rounded cells.

**Lumen formation.** The formation of a lumen in the Wolffian duct has been said to start in the chick at the 14- to 15-somite stage. Beginning as a hollowing out in the region of the fifth to sixth somite, the process has progressed the entire length of the duct in the 16- to 18-somite embryo. Thus the undifferentiated mesoblast cells become tubular epithelium as a result of their circular arrangement around a central lumen (*Mihalkovics, 1885*). The lumen was first noticed by Felix (1906) in the 20- to 22-somite chick embryo. This author stated that the lumen is at first discontinuous (but not in a segmental fashion) throughout the entire length of the excretory duct. Later the parts of the lumen join, forming a continuous cavity within the growing duct. Lumen formation by flattening of the sides of the cord and subsequent arching of these edges until they meet and fuse (*Waldeyer, 1870, p. 115*), has never been confirmed.

**Relation to the cloaca.** The growing end of the Wolffian duct reaches the dorsolateral surface of the cloaca (Fig. 298-E) in chicks incubated 63 to 66 hours, that is, in embryos of 32 to 37 somites (*Mihalkovics, 1885; Schreiner, 1902; Abdel-Malek, 1950*). In the 40-somite chick neither duct has quite fused with the cloaca (*Boyden, 1922a*).

On the fourth day of incubation, the epithelium between the ventral side of the duct and the contiguous dorsolateral protuberance of the cloaca degenerates and connection is established. Thus the opening is in the dorsolateral part of the cloaca (*Mihalkovics, 1885*). The duct has been seen to open into the cloaca in the 35-somite embryo incubated 72 hours (*Abdel-Malek, 1950*). The opening into the cloaca has been noted at the level of the thirty-third to thirty-fourth somite in the chick (*Sedgwick, 1880; Schreiner, 1902*), and at the level of the thirty-fourth to thirty-fifth somite (*Schreiner, 1902*) in the duck (*Anas platyrhynchos*) and the gull (*Larus ridibundus*).

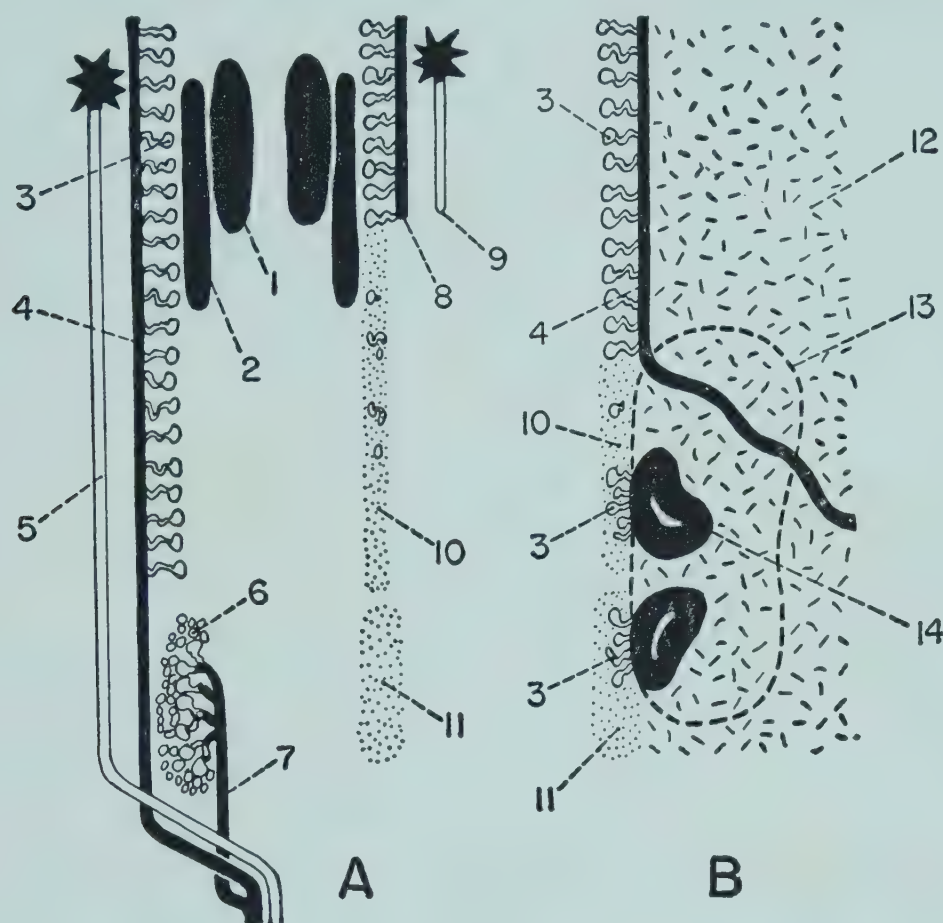
#### *Experimental Determination of Physiological Capacity of Ducts*

It has been shown experimentally that the ducts are important inducers. Complete removal of the duct provides some conception of its influence on related structures. Without the growth stimulus provided by the duct, mesonephric tubules, metanephric duct and tubules, and Müllerian ducts fail to develop.

**Experiments on differentiating capacity.** The duct is self-differentiating and grows without the addition of cells from surrounding ectoderm or



mesoderm (Gruenwald, 1937). Its growth is the result of the multiplication of its own cells. A transverse cut on one side of an 8- to 12-somite chick embryo prevents the growth of the duct beyond the cut, while growth continues on the unoperated side (Waddington, 1938). Seven- to eleven-somite chick embryos in which the Wolffian duct was cut exhibited self-differentiation of the duct. Above the wound, the duct is dilated from fluid accumula-



**Fig. 299.** Diagram showing developmental relationships of urogenital organs. Structures shown in solid black are capable of developing independently. Outlined structures are dependent on an inductor for normal development. (Redrawn after Gruenwald, 1952.)

A, left side of diagram shows normal developmental relationships, right side shows effect of cutting Wolffian duct, thus removing inducing stimulus; Müllerian duct, mesonephric structures, and metanephros fail to develop normally below level of cut; B, abnormal development in response to a graft of nonnephrogenic tissue which contains some nervous tissue instead; the latter induces mesonephros differentiation in both mesonephrogenic and metanephrogenic tissue.

1, adrenal cortex; 2, gonad; 3, mesonephric tubule; 4, mesonephric (Wolffian) duct; 5, Müllerian duct; 6, metanephric tubule; 7, ureteric bud; 8, cut end of Wolffian duct; 9, end of growth of Müllerian duct; 10, mesonephrogenic tissue; 11, metanephrogenic tissue; 12, body wall of host; 13, outline of area of graft; 14, nervous tissue.

tion, whereas below, it is shorter and more slender. Although the duct can elongate by its own growth, it also receives contributions from pronephric tubules (Kumé, 1941).

**Role as an inducing agent.** The Wolffian duct plays a vital role in the development of certain parts of the urogenital tract. In the absence of the duct or the ureteric bud (which becomes the metanephros) (Fig. 299-A), neither the mesonephric or metanephric portions of the nephrogenic tissue



differentiates into tubules (Boyden, 1927; Gruenwald, 1937). Explants of chick embryo nephrogenic tissue in chorioallantoic cultures may develop into mesonephros providing Wolffian duct material is present (Hunt, 1931a; Willier and Rawles, 1931b). In the absence of the Wolffian duct or a branch, nephrogenous tissue fails to differentiate into kidney elements in every case (Danchakoff, 1924b).

It has been shown, however, that partial differentiation of mesonephric tissue can occur even in the absence of the inducing action of the Wolffian ducts (Waddington, 1938; Gruenwald, 1942b; Kumé, 1943). Stimulation by nervous tissue has been suggested as the cause of the partial differentiation of mesonephros in the absence of the duct (Gruenwald, 1952). The initial appearance and normal development of the primitive oviduct, the Müllerian duct, are dependent on the Wolffian duct (Gruenwald, 1937, 1941a) for evocation and stimulation.

In addition to evoking the normal development of certain organs, the presence and function of the Wolffian duct is a necessary step in the normal development of certain other structures such as the allantois, cloacal diverticula, and cloacal sinus (Boyden, 1924).

In grafts of portions of the ureter of chick embryos of 12 to 14 days' incubation, the first rhythmic contractions of the muscular layers appear after about 1 hour. The location of the initial contractility is well defined; it is in the longitudinal muscle fibers of the metanephric segment. In chorioallantoic transplants, the Wolffian ducts of males reach a high degree of spontaneous differentiation, whereas the Wolffian ducts of females, under similar conditions, undergo no further differentiation (Staudacher-Dalle Aste, 1941).

### The Mesonephros or the Intermediate Kidney

The mesonephros has its origin in the intermediate cell mass. Unlike the pronephros, the mesonephros is not entirely self-differentiating; the inducing action of the Wolffian duct is necessary for normal development, although, in the absence of the Wolffian duct, nervous tissue may evoke a limited degree of mesonephros development.

The anterior mesonephric tubules, extending from the thirteenth or fourteenth somites to the twentieth somite, are nonfunctional and rudimentary. They occur in the same region as the posterior pronephric tubules but instead of being blind, they open into the coelom through nephrostomes. Tubules posterior to the twentieth somite have a well-formed glomerular apparatus, and do not open into the coelom.

Mesonephros formation occurs rapidly, with the tubules at a given level appearing simultaneously. This type of development is in contrast to the gradual caudad progress of the pronephric tubules. The mesonephric duct acts as the main excretory channel. Primary tubules, three or four to a



somite, arise first in the most ventral nephrogenous tissue. Secondary and tertiary tubules are subsequently formed more dorsally in each somite. In the chick, mesonephric function begins approximately on the fifth day and continues until the eleventh day of incubation.

### *Structural Development*

Grafts have shown that mesonephric tissue becomes destined as such early in embryonic life and well before visible differentiation occurs. All parts of the kidney are derived from the intermediate mesoderm. The first mesonephric tubules to form are the most anterior; they are rudimentary and soon degenerate. Tubules located posterior to the twentieth somite are fully formed and number five or six per somitic segment. Secondary and tertiary tubules differ only in time or origin. Whereas pronephric tubules have only rudimentary external glomeruli, mesonephric tubules are generally associated with internal glomeruli as well. In its gross appearance, the mesonephros is an elongated, paired organ, consisting mainly of tubules, lying behind the peritoneum. The mesonephros functions during embryonic life and degenerates rapidly shortly before the time of hatching.

**Embryonic potentiality.** Mesonephric tissue is apparently determined before either Hensen's node or the head-process appears. Hoadley (1926d) found that mesonephric potency exists in the 10-hour chick blastoderm. Grafts taken from the level of the anterior half of the primitive streak formed convoluted, columnar, secreting tubules, cuboidal collecting tubules, and large well-formed glomeruli in a typical capsule; in some cases, glomeruli and tubules were directly connected. It can be pointed out, however, that in the past this anterior level of the primitive streak was considered as the probable equivalent of the node (Willier and Rawles, 1931b).

Subsequent work with chorioallantoic grafts of isolated levels of the chick blastoderm has shown that mesonephros can be formed from tissue of a 12- to 18-hour blastoderm, if the graft is taken through the level of Hensen's node (Hunt, 1931a). One-somite embryos also have mesonephric potentialities in grafts taken through the node level. Hunt showed that mesodermal structures form in grafts from the node and primitive streak levels of blastoderms in the advanced head-process stage. The capacity of the node level of the blastoderm to differentiate mesonephros gradually increases as the age of the blastoderm increases and thus as the node moves posteriorly (Willier and Rawles, 1931b). In the head-process stage, the mesonephros-forming area of the blastoderm exhibits a mediolateral gradient in developmental potency, with the highest intensity in the center and a much lower percentage of growth and survival of grafts from right to left sides (Willier and Rawles, 1935).

**Mesonephric differentiation.** The mesonephric blastema from which the mesonephros is derived is the portion of the intermediate cell mass



extending from the thirteenth or fourteenth somites to the thirtieth somite. Differentiation and subsequent development of the mesonephros was at first shown to occur only in the presence of the evocator, the Wolffian duct (*Danchakoff, 1924b; Boyden, 1927; Hunt, 1931a; Gruenwald, 1937*). More recent work suggests a partial independence of mesonephric tissue which enables it to develop in the absence of a physiological inducer (*Waddington, 1938; Gruenwald, 1942b; Kumé, 1943*). Nervous tissue probably also provides a stimulus (cf. Fig. 299-B) to nephrogenic differentiation (*Gruenwald, 1942b*). That the Wolffian duct is the specific initiator for the mesonephros was indicated in any instance where it induced mesonephric formation in the metanephric blastema. This suggests a general nephrogenic potency which responds to specific stimulation (*Gruenwald, 1942b*).

**Formation of tubules.** The first development of the mesonephric tubules begins anteriorly and proceeds caudad. The mesonephros is present along with the pronephros in somites 9 to 15, both having had the same origin, their primordia being at first continuous, but the tubules differentiating individually (*Abdel-Malek, 1950*). Anterior to the twentieth segment, the intermediate cell mass is at first continuous with the peritoneal epithelium, then separates from it and quickly breaks up into the rudiments of the tubules. The remaining tubules arise from blastema which had no connection with the peritoneum and never possessed nephrostomes (*Sedgwick, 1880*). This partial continuity with the peritoneum anteriorly led to the erroneous conclusion that tubules arose from the peritoneum and later became connected with the Wolffian duct (*Fürbringer, 1878*).

Mesonephric tubules first arise as condensations of cell masses (Fig. 300-A). These are dense clumps of cells as far posterior as the twentieth somite of the 30-somite embryo, less discrete condensations more caudally. The most anterior vesicles represent a complete mesonephric tubule. In the 35-somite embryo, the anterior tubules have fused with the duct, and their lumina are continuous. The posterior tubules meanwhile are nascent, lying median to the duct and close to it.

Tubules anterior to the twentieth somite form Malpighian corpuscles by flattening of the median portion of the vesicles, leaving a slitlike lumen. This flat, two-layered disc then becomes concave, with the inner wall thick and the outer wall thin. At first the vesicle is nearly oval (Fig. 300-C), then it becomes elongate, appearing horizontal in the stage of the first curvature (Fig. 300-D). Following a vertical rotation, the second curve appears (Fig. 300-G) and lastly, the lumen opens into that of the collecting duct (Fig. 300-H).

From this is derived the S-shaped primary tubule (Fig. 300). Primary tubules do not become functional (*Sedgwick, 1880*).

The principal mesonephric tubules lie between the twentieth and thirtieth somites. The first of these to appear are 3 or 4 in the ventral por-



tion of each of the anterior somites. After the seventy-second hour, tertiary tubules, five or six in each somite, are formed in the undifferentiated tissue from the twentieth to the thirtieth somite.

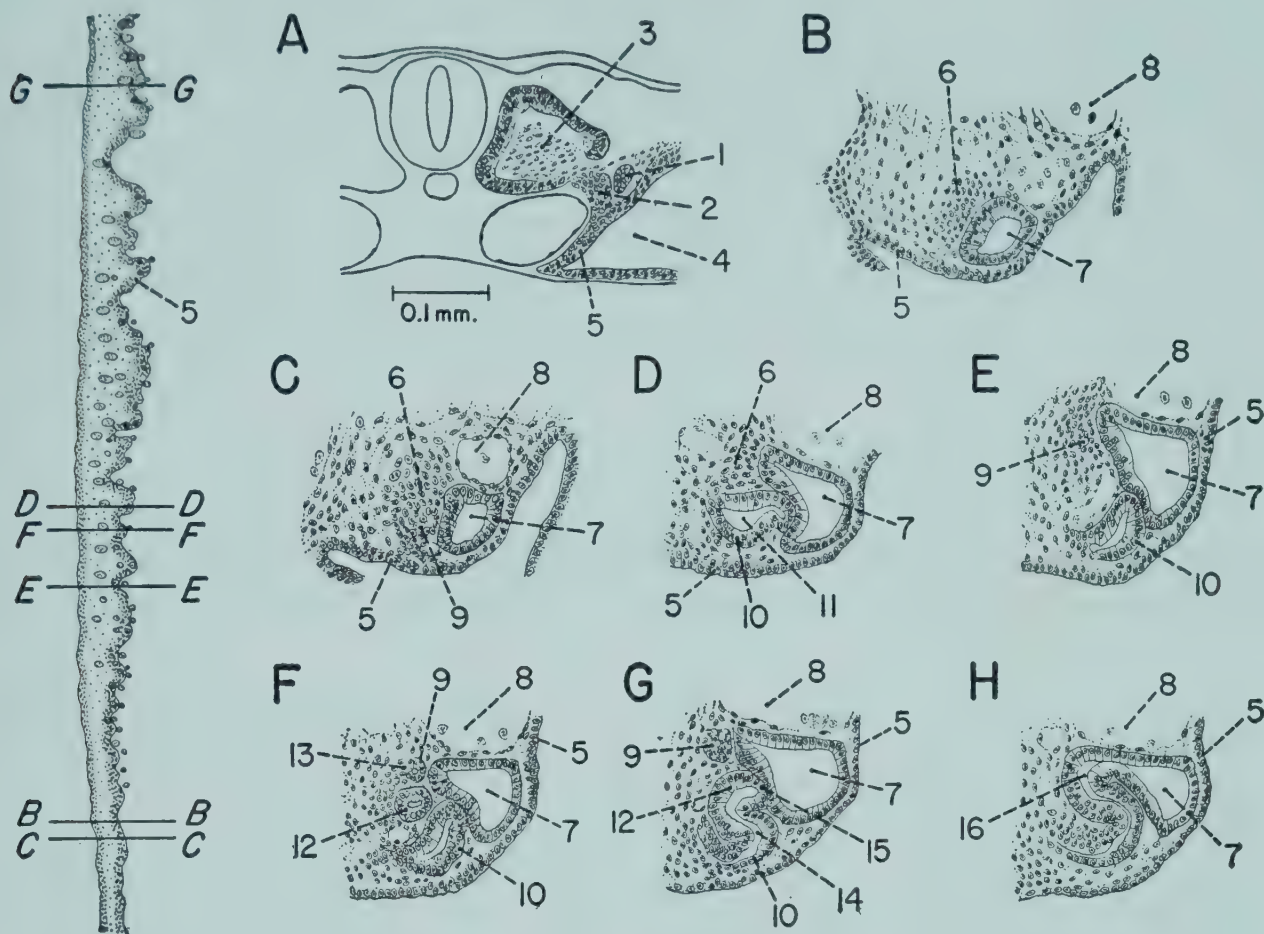


Fig. 300. Stages in the development of the mesonephric tubule in the avian embryo. (Redrawn with modifications A, after Abdel-Malek, 1950; B to H, after Schreiner, 1902.)

At left: diagrammatic view of mesonephric tissue taken at a plane which forms a  $45^\circ$  angle opening ventrally to the sagittal plane, showing levels at which sections B to G were taken; 45-somite duck (*Anas platyrhynchos*) embryo.

A, section of 40-hour chick embryo taken at level of anterior portion of somite 14, showing relationship of pronephric duct to mesonephric primordium; B, concentration of nephrogenous tissue, in the 45-somite duck embryo; C, radial arrangement of inner nephrogenous cells, in the same; D, primary mesonephric tubule after slight elongation toward Wolffian duct and flattening of lumen, in the same; E, primary tubule lumen becoming more slitlike, secondary tubule forming dorsal to primary tubule, in the same; F, tertiary tubule forming dorsal to secondary tubule, in the same; G, secondary tubule joined to primary tubule to form continuous lumen and early S-shaped tubule, preparatory to opening into Wolffian duct, in the same; H, stage immediately before direct connection of lumen, in the same. A, in scale; B to H,  $\times 75$ .

1, pronephric duct; 2, mesonephric primordium; 3, somitic mesoderm; 4, coelom; 5, coelomic epithelium; 6, nephrogenous tissue; 7, Wolffian duct; 8, cardinal vein; 9, radial arrangement of some nephrogenous cells; 10, wall of primary mesonephric tubule; 11, lumen; 12, wall of secondary mesonephric tubule; 13, tertiary mesonephric tubule; 14, S-shaped lumen formed by joining of primary and secondary tubules; 15, cell barrier growing thin; 16, mesonephric tubule lumen just before open connection with Wolffian duct is established.

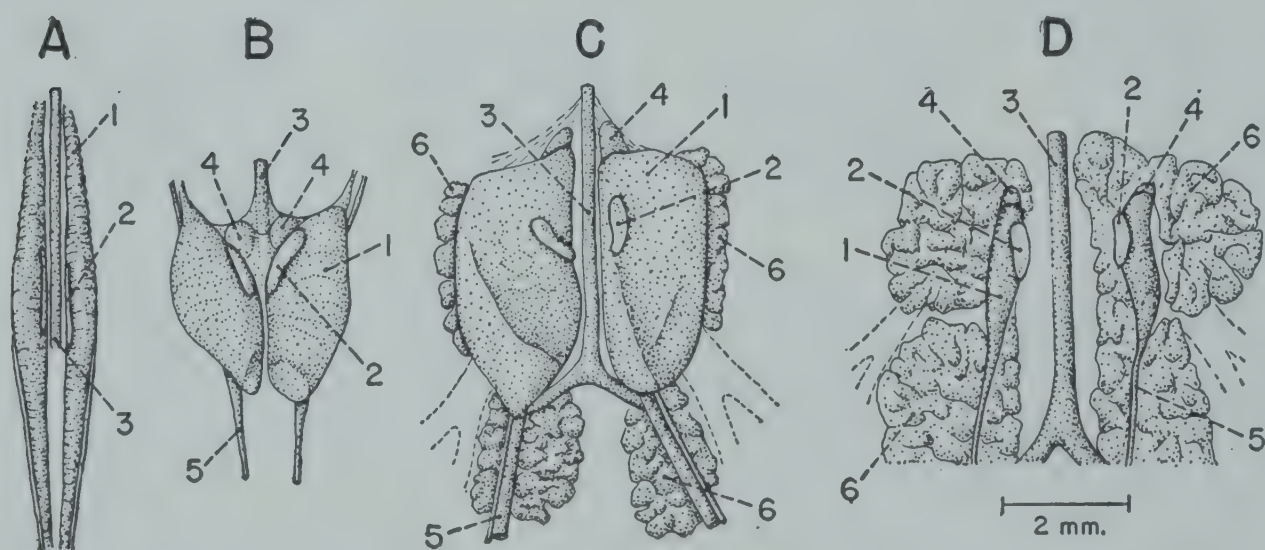
**Formation of glomeruli.** The formation of glomeruli was studied in detail by Davies (1950), who used the duck embryo (*Anas platyrhynchos*). At 96 hours the internal glomeruli appeared as cell clumps adherent to the



walls of the Wolffian duct. In one case, they extended almost to the cranial end of the duct. The 4.5-day duct embryo had at least eight well-differentiated internal pronephric glomeruli enclosed within Bowman's capsules and forming true Malpighian bodies (Davies, 1950). The rapid fibrosis which accompanies degeneration affects the external glomeruli and also involves the Malpighian bodies closest to them. The pronephric glomeruli and the internal mesonephric glomeruli fuse at the level where they overlap (cf. Fig. 298-B), and this zone persists in the duck until about the eighth incubation day. This intermediate fused zone observed by Davies (1950) is mainly rudimentary in the chick but probably functional in the duck.

### *Gross Anatomy of the Intermediate Kidney*

During the first quarter of the incubation period, the general configuration of the mesonephros is elongated (Fig. 301-A), later becoming roughly



**Fig. 301.** Four stages in the development of the avian male urogenital system. (Redrawn and modified after Stampfli, 1950.)

A, general appearance of avian mesonephros during first quarter of incubation period, showing position of gonads on median surface of mesonephros; B, second period of incubation marked by change in mesonephros shape and size, growth of testes, and appearance of adrenal; C, height of mesonephros development during third quarter of incubation, first appearance of metanephros; D, fourth quarter of incubation in which mesonephros has partially degenerated, metanephros and testes have increased in size. All in scale.

1, mesonephros; 2, gonad; 3, aorta; 4, adrenal gland; 5, Wolffian duct; 6, metanephros.

oval (Fig. 301-B), then truncated (Fig. 301-C), and finally, during degeneration, shorter and thinner (Fig. 301-D).

In a study of the mesonephros, Stampfli (1950) made gross measurements of this organ in several species of birds, as shown in Table 14. These figures are compared with the length of the body cavity from the level of the anterior appendages to the cloaca.



TABLE 14

Dimensions of Mesonephros in Different Species of Birds \*

Species	Incubation Age	Mesonephros		Body Cavity
	(days)	Length (mm.)	Width (mm.)	(mm.)
Chick, <i>Gallus gallus</i> (Incubation period, 21 days)	5	3.5	0.5	
	8	5.2	2.2	7.5
	14	6.5	2.8	15.0
	19	4.0	1.9	40.0
Swift, <i>Apus melba</i> (Incubation period, 20 days)	5	3.5	0.5	
	7.5	2.4	1.1	4.5
	13	2.5	1.4	9.0
	18	2.2	1.2	18.5
European blackbird, <i>Turdus merula</i> (Incubation period, 14 days)	3.5	3.5	0.5	
	6	3.2	1.3	6.0
	9	2.9	1.6	10.5
	12	2.9	1.2	17.8

\* After Stampfli (1950).

*Histology of the Nephric Unit*

The mesonephros can be conveniently separated into subdivisions. The nephric unit, generally referred to as the tubule, starts at the glomerulus and its surrounding capsule, and empties directly into the main tubule. This long, highly coiled kidney unit is separated into a proximal portion and a distal tubule, with several intermediate transitional "segments."

An extensive investigation of the histology of the mesonephros in eleven species of birds was made by Stampfli (1950). The chicken (*Gallus gallus*), the European blackbird (*Turdus merula*), and the alpine swift (*Apus melba*) were studied in great detail. Material was also examined from embryos of the tern (*Sterna hirundo*), the European quail (*Coturnix coturnix*), the European swift (*Apus apus*), the starling (*Sturnus vulgaris*), the sparrow (*Passer domesticus*), the blackcap (*Sylvia atricapilla*), the European martin (*Delichon urbica*), and the zebra parakeet (*Melopsittacus undulatus*). The histology of the mesonephros is best observed during the third quarter of the incubation period. This is the period of maximum development of the five major regions of the mesonephric tubule: the glomerulus, the proximal tubule (main segment), the transition segment, the distal tubule (midsegment), the connecting segment, and the



mesonephric duct (Fig. 302). Maximum function occurs between the tenth and fifteenth days of incubation in the chick; between the ninth and thirteenth days in the swift (*Apus melba*); and between the seventh and eleventh days in the European blackbird (*Turdus merula*).

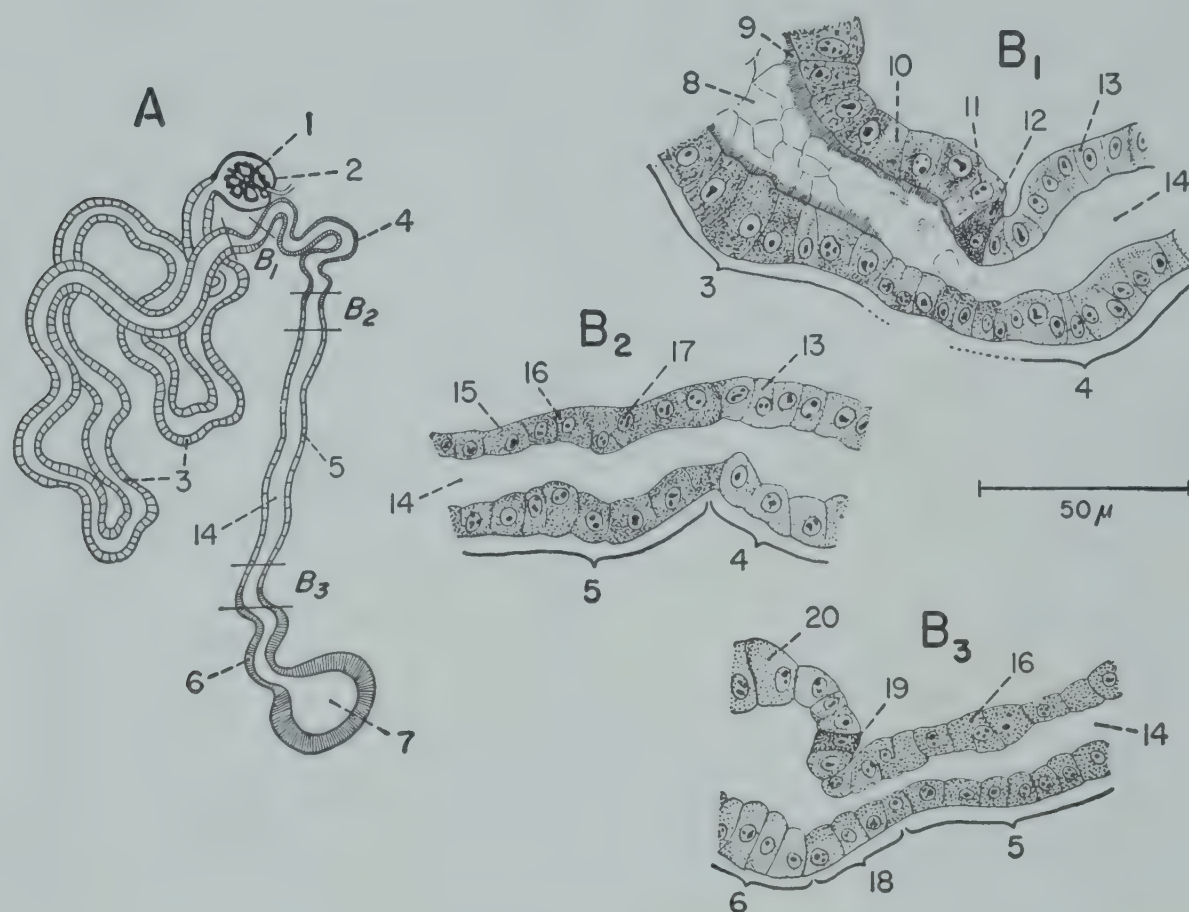


Fig. 302. Histology of the mesonephric tubule, as seen in common in the chick, the alpine swift (*Apus melba*), and the European blackbird (*Turdus merula*) during the third quarter of incubation. (Redrawn with modifications after Stampfli, 1950.)

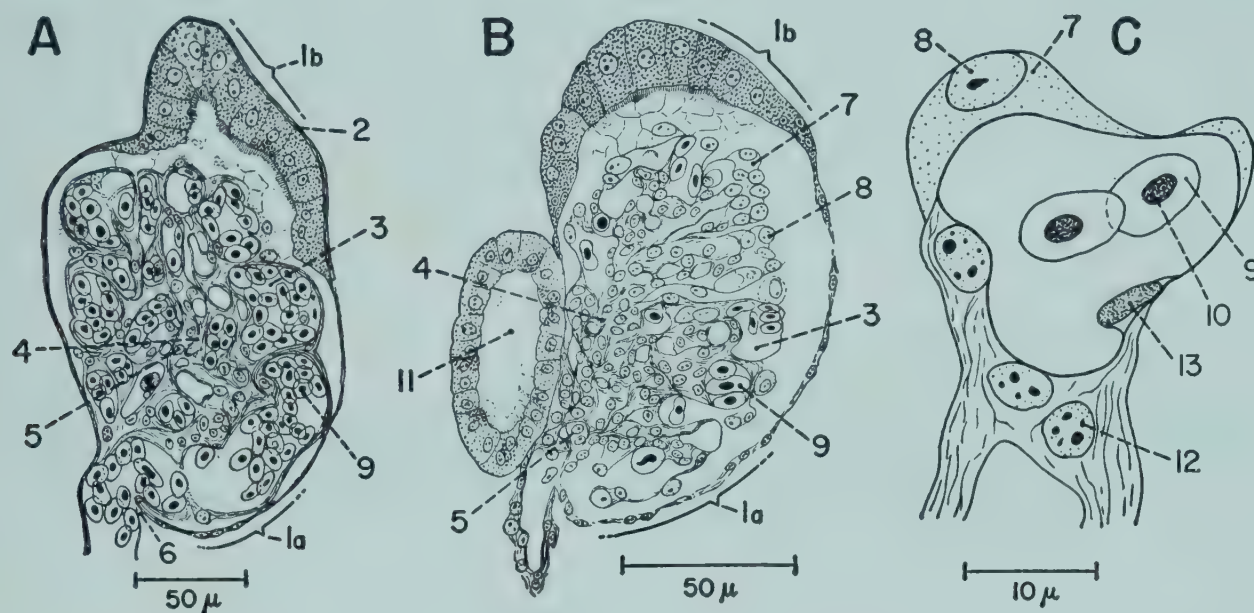
A, diagrammatic representation of a mesonephric tubule giving location of portions shown in detail; B<sub>1</sub>, main segment (proximal tubule) and abrupt change to transition segment, comparable to loop of Henle (in scale); B<sub>2</sub>, transition segment and rather abrupt change to midsegment, comparable to distal tubule (in scale); B<sub>3</sub>, midsegment at point of junction with connecting segment. B<sub>1</sub> to B<sub>3</sub>, in scale.

1, glomerulus; 2, Bowman's capsule; 3, main segment of mesonephric tubule; 4, transition segment; 5, midsegment; 6, connecting segment; 7, mesonephric duct; 8, alveolar lumen; 9, brush border; 10, granular cytoplasm of cuboidal epithelial cell; 11, epithelial cell nucleus with two nucleoli; 12, a single transition cell; 13, columnar epithelial cell of transition segment; 14, empty lumen; 15, basement membrane; 16, heavily granular cytoplasm of cuboidal epithelium; 17, rounded nucleus with two nucleoli; 18, gradual transitional cell; 19, abrupt transitional cell; 20, lightly granular columnar epithelial cell.

**The glomerulus.** The glomerulus, as described by Stampfli (1950), is round to elliptical in shape and relatively large: its diameter is 122 microns in the chick, 104 microns in the swift (*Apus melba*), 108 microns in the European blackbird (*Turdus merula*), and 72 microns in the sparrow (*Passer domesticus*). There is a distinct space between the glomerulus and Bowman's capsule (Fig. 303-A and B). The capsule is composed of connective tissue, and its basal membrane is not always apparent. The glom-



erulus leads directly into the main tubule. The structure of the mesonephric glomerulus is very similar to that of the metanephric glomerulus. The capillary is extensively coiled around a central compact mass of connective tissue. The connective tissue nuclei are closely packed, small, varied in form, and contain a large quantity of chromatin material. The connective tissue mass consists of fibrocytes which migrate into it at any early stage of vascularization. The basal membrane is always distinct and optically homogeneous; it is not divided into two membranes. The capil-



**Fig. 303.** Histology of Malpighian corpuscles of the mesonephros in two species of birds. (Redrawn with modifications after Stampfli, 1950.)

A, Malpighian corpuscle of the European blackbird (*Turdus merula*) during latter half of embryonic period; B, Malpighian corpuscle of the chick (*Gallus gallus*) at a similar age; C, capillary and pericyte cell in detail. All in scale.

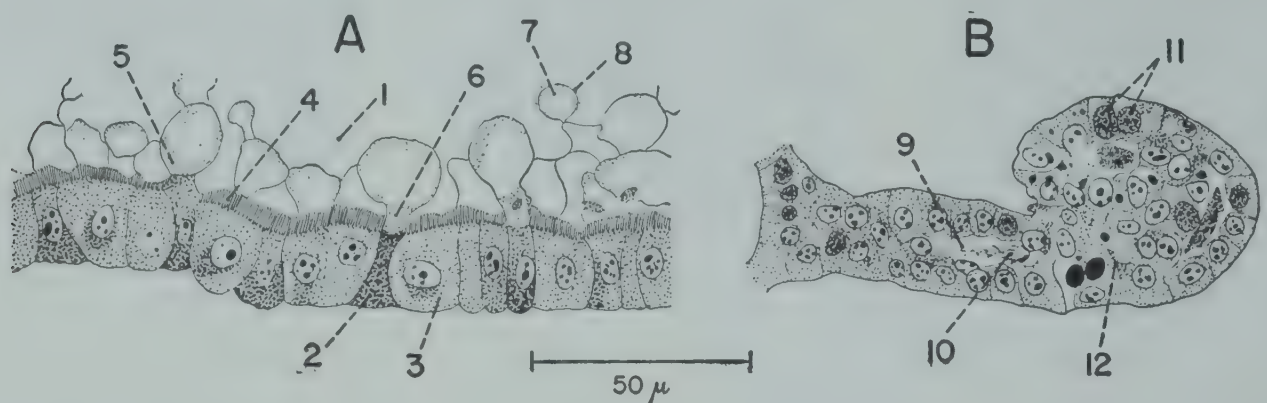
1a, proximal portion of Bowman's capsule; 1b, distal portion of Bowman's capsule; 2, basement membrane of Bowman's capsule; 3, lumen of glomerular blood vessel; 4, central mass of compact connective tissue; 5, fibrocyte nucleus; 6, transition between capsule and mesonephric tubule; 7, pericyte; 8, pericyte nucleus; 9, erythrocyte; 10, blood cell nucleus; 11, lumen of mesonephric tubule; 12, connective tissue nucleus; 13, endothelial cell nucleus.

lary lumen is lined by endothelial cells which have elongated, chromatin-packed nuclei. Their cytoplasm is clear, weakly granular, and often indistinct. The nuclei are far apart, and there are no apparent cell outlines. There is some question about the actual structure and relation of the glomerular pericytes, "Deckzellen" (Fig. 303-C), which are large, loose cells forming a spongy covering over the capillaries. The layer of pericytes is connected with the capsular epithelium. The pericyte has a large, rounded nucleus which is clear because of the small quantity of chromatin. There are never more than two nucleoli.

**The proximal tubule.** The main or proximal segment of the mesonephric tubule leads away from the glomerulus (Fig. 302-A) as described by Stampfli (1950). It has an average diameter of 45 microns in the chick



and 40 microns in the swift (*Apus melba*) and the European blackbird (*Turdus merula*). The cuboidal epithelium is composed of distinctly outlined cells 14 to 18 microns in height (Fig. 302-B<sub>1</sub>). The single, rounded nucleus may be central or close to the basal end and contains one to three nucleoli. The cytoplasm is uniform, except after the onset of degeneration, when marked granulation occurs and a supranuclear zone can be distinguished. The characteristic brush border of nonmotile cilia in this segment is 2 to 4 microns high. The epithelial cells rest on a straight, distinct basement membrane. The lumen is typically alveolar, giving the effect of a network which is in contact with points projecting from the epithelial cells. The origin of the network may be in the condensation of the vesicles which secretory cells give off into the lumen (Fig. 304-A).



**Fig. 304.** Comparison of function and degenerating mesonephric proximal tubule in the European blackbird (*Turdus merula*). (Redrawn with modifications after Stampfli, 1950.)

A, epithelial cells of main segment showing secretory activity during seventh to tenth day of incubation; B, portion of main segment undergoing degenerative changes; this is the general picture from a late stage of the incubation to the early postembryonic period. Both in scale.

1, lumen; 2, narrow, heavily granular, secreting cell; 3, nonsecretory, less granular, cuboidal epithelial cell; 4, brush border; 5, secretory vesicle interrupting brush border; 6, granular stalk connecting vesicle to cell; 7, detached vesicle contributing to alveolar appearance; 8, persistent granules; 9, remains of lumen; 10, deposit of dark pigment granules; 11, deeply staining nuclei of proximal tubule cells; 12, degeneration of cell membranes.

There is an abrupt transition from the main segment to the transitional segment (cf. Fig. 302-A). The cuboidal epithelium is suddenly replaced by cylindrical epithelial cells. This phenomenon is in contrast both to the change to squamous epithelium in the homologous structure (loop of Henle) in the mammal and to the continuation of cuboidal epithelium in the corresponding segment of the avian metanephros. Between the last cell of the main segment and the first cylindrical cell there may be one heavily granular cell (cf. Fig. 302-B<sub>1</sub>). The tubule diameter has decreased to 32 microns and the epithelium to a height of 10 microns. The round to elliptical nuclei may be central or basally situated; they contain one to three



nucleoli. In the chick, the cytoplasm is less granular in this segment and appears lighter than that of the main segment. Cell boundaries are distinct, and a well-defined, straight basement membrane underlies the cells. These epithelial cells may bulge into the lumen, which is empty and clear in this segment.

*The distal tubule.* The midsegment or distal tubule is clearly distinguishable from the transition segment (cf. Fig. 302-A). Its diameter is uniform throughout, averaging 28 microns in all species studied. The cuboidal epithelium, 8 microns high, rests on a well-defined basement membrane (cf. Fig. 302-B<sub>2</sub>). The cells bulge slightly into the lumen, and the division between individual cells is not always definite. The round nucleus has one to three nucleoli and lies in the center of the cell. The average nuclear diameter is 6 microns. The cells are more granular than in the transition segment, and the granulation is uniform throughout. The lumen has no inclusions.

The connecting segment is very reduced, short, and only slightly coiled (cf. Fig. 302-A). It appears late in tubule development. The change from the midsegment may occur abruptly, or gradually (cf. Fig. 302-B<sub>3</sub>), with the epithelium increasing in height to an average of 10 microns and the lumen expanding to an over-all average diameter of 60 microns. The epithelial cells are cylindrical, narrow, and bulge into the lumen. The nucleus is typically basal and elongated. The cytoplasm is less granular than in the preceding segment. The basement membrane and cell membranes are distinct, and the lumen is empty.

The collecting (mesonephric) duct develops its characteristic appearance in the third quarter of incubation (cf. Fig. 302-A). Its walls are high columnar epithelium with very narrow cells and the elliptical nuclei are located at the base of the cells. The cells bulge into the lumen and are separated by distinct cell membranes. The cytoplasm is clear and has no inclusions. The principal distinction from the connecting segment is in the higher epithelium (20 microns) and the narrower cells, and in the fact that the nuclei lie closer to one another. The lumen is larger.

### *Mesonephric Function*

The onset of mesonephric function probably occurs during the fifth day of the chick's incubation period. Evidence for this conclusion is provided by the occurrence of hydronephrosis in cut ducts and by the movement of dyes against a concentration gradient. Dyes are picked up by cells of the proximal convoluted tubule in a manner of minutes after injection. Lowering the pH does not affect dye excretion activity, but lowering the temperature renders the epithelium permeable in both directions. Maximum function is reached around the eleventh day and this is followed by signs of degeneration. Cessation of function progresses in a caudal direction and



occurs shortly before hatching, at approximately the same time that the metanephros begins to function. Details of renal function are not well known, nor is there any information on abnormalities of renal function or on morphological renal damage produced by experimental methods. Degenerative changes involve shrinkage of tubules and glomeruli and eventual conversion to connective tissue. Degeneration can be induced by certain chemical substances.

**Initiation of functional activity.** The beginning of functional activity has been determined largely by experiments with dyes. The first sign of dye being picked up by tubule cells and passed against the concentration gradient into the tubule lumen can be interpreted as the beginning of function. One of the earliest observations of this occurrence was made by Bakounine (1895), who noted the appearance of indigo carmine in the tubule lumen of 5-day and older chicks. Trypan blue injected in the chick air sac is picked up by the mesonephros as soon as the respiratory vascular net reaches the air sac, that is, on the fifth day (Atwell and Hanan, 1926; Hurd, 1928). Other studies based on trypan blue absorption place the beginning of function at 4 days (Hanan, 1927) and at 5 days (Schneider, 1940). Chambers and Kempton (1933) first observed function at 4.5 days, and Boyden (1924) also reached this conclusion on the basis of his studies of hydronephrosis of cut Wolffian ducts. Pirner (1949) claimed that the initiation of function could be gauged by the time that innervation occurred. This he first noted on the fifth day in the embryo chick. From histological evidence, Stampfli (1950) concluded that function begins on the sixth day in the chick, the seventh day in the swift (*Apus melba*), and the fifth day in the European blackbird (*Turdus merula*). The indications in the latter three species are the attainment of completely differentiated glomeruli and main segments of mesonephric tubules, the large size of capillaries and their full blood content, and the first appearance of granular, columnar, secretory cells in the main segment of the tubule (cf. Fig. 304-A). The appearance of cloudy fluid, presumably in the allantois, was used as an indication of functional activity (Kuo, 1932b). This commenced on the thirteenth day of chick incubation and the percentages of occurrence on several successive days are given in the accompanying table.

Age of Chick Embryo (days)	Appearance of Cloudy Fluid in Allantois (per cent)
13	23.56
14	48.87
15	21.80
16	5.76

The glomerular volume (Fig. 305) and number (Fig. 306) are directly correlated with the pattern of development and degeneration in the meso-



nephros and should therefore serve as indicators of functional activity. It can be seen that the peak in both number and volume occurs at approximately the twelfth to fourteenth day in the chick and at a corresponding stage of incubation in the European blackbird (*Turdus merula*) and the swift (*Apus melba*). During the period when uric acid is excreted in progressively larger amounts, the secreting portion of the mesonephric tubule is the fastest growing part. From the fifth to the fourteenth day of incubation, the entire Wolffian body of the chick shows a twelvefold increase in volume. The number of glomeruli does not change markedly, but they

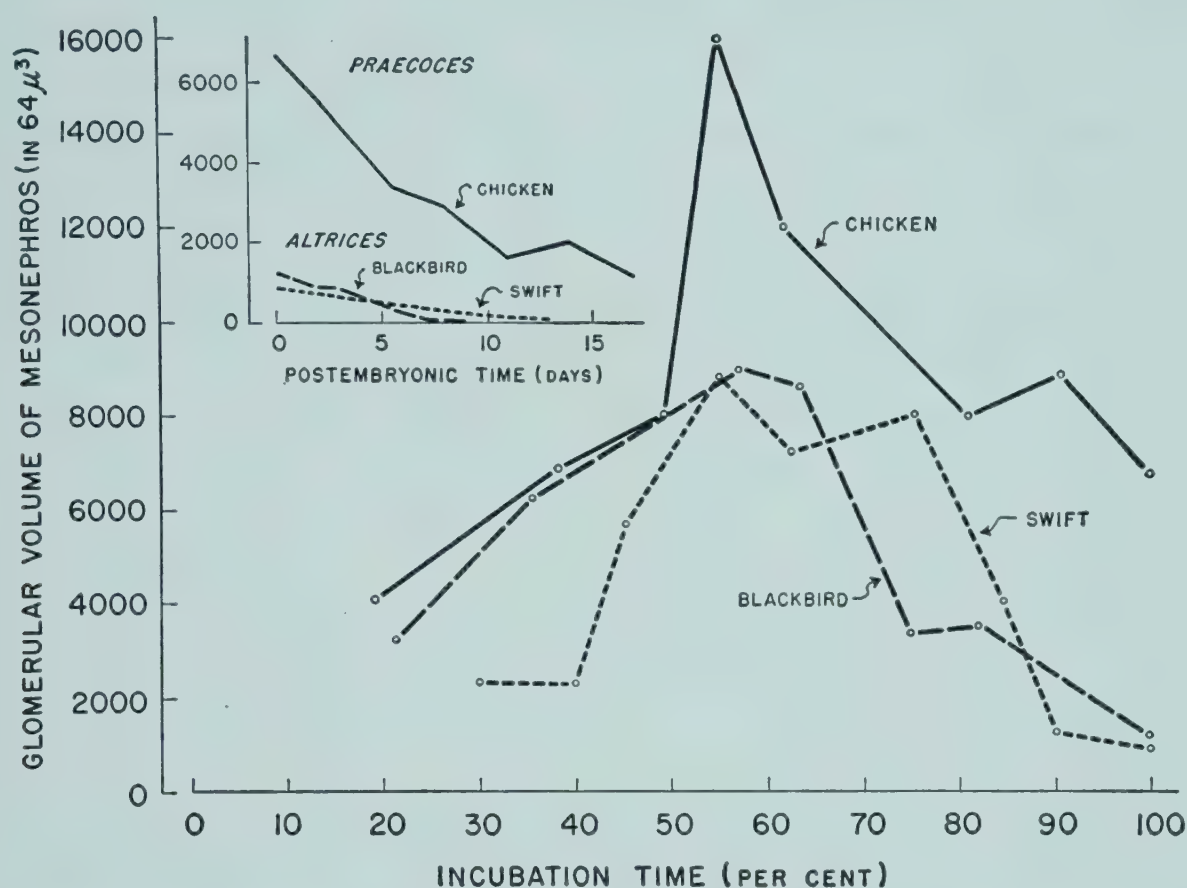


Fig. 305. Volume of mesonephric glomeruli in embryos of the chicken (*Gallus gallus*), the European blackbird (*Turdus merula*), and the alpine swift (*Apus melba*); the curves show that the period of greatest glomerular volume coincides with the time (third quarter of incubation period) when mesonephric function is at a peak. (Modified after Stampfli, 1950.)

*Insert:* curves showing the relatively slower postembryonic reduction in glomerular volume in a precocial bird (chicken) as compared with altricial species (swift, European blackbird).

double their volume. The volume of the collecting tubules increases ten times, that of the secretory tubules, thirty-three times (Boyden, 1931).

**Reactions to dyes.** Kidney tissues exhibit a certain specificity in experiments with dyes. Phenol red, for example, is picked up only by the epithelium of the proximal tubule, and thus it serves as a test for activity of this tissue. Using various chemical substances, Chambers and Cameron (1932) found that the columnar cells of the proximal convoluted tubule picks up sulphonephthalein, cresol red, phenol red, and chlorophenol red; and that bromthymol blue and bromcresol purple are more or less toxic to



the tubule cells. Trypan blue, when administered *in vivo*, appears in high concentrations in the cells of the proximal segment; *in vitro*, it collects in masses outside the cells. This differential behavior is probably the result of an uninterrupted reabsorption current *in vivo* and the lack of this current and consequent accumulation in the lumen *in vitro* (Deen and Haan, 1940). Trypan blue was not found to be stored in Bowman's capsule by Hanan (1927) but was observed in its inner cells by Hurd (1928). Large amounts of the dye have been seen in macrophages associated with the basement membrane of the mesonephric tubules (Hurd, 1928). Ferrocyanide has been used as an indication of glomerular activity (Gersh, 1937).

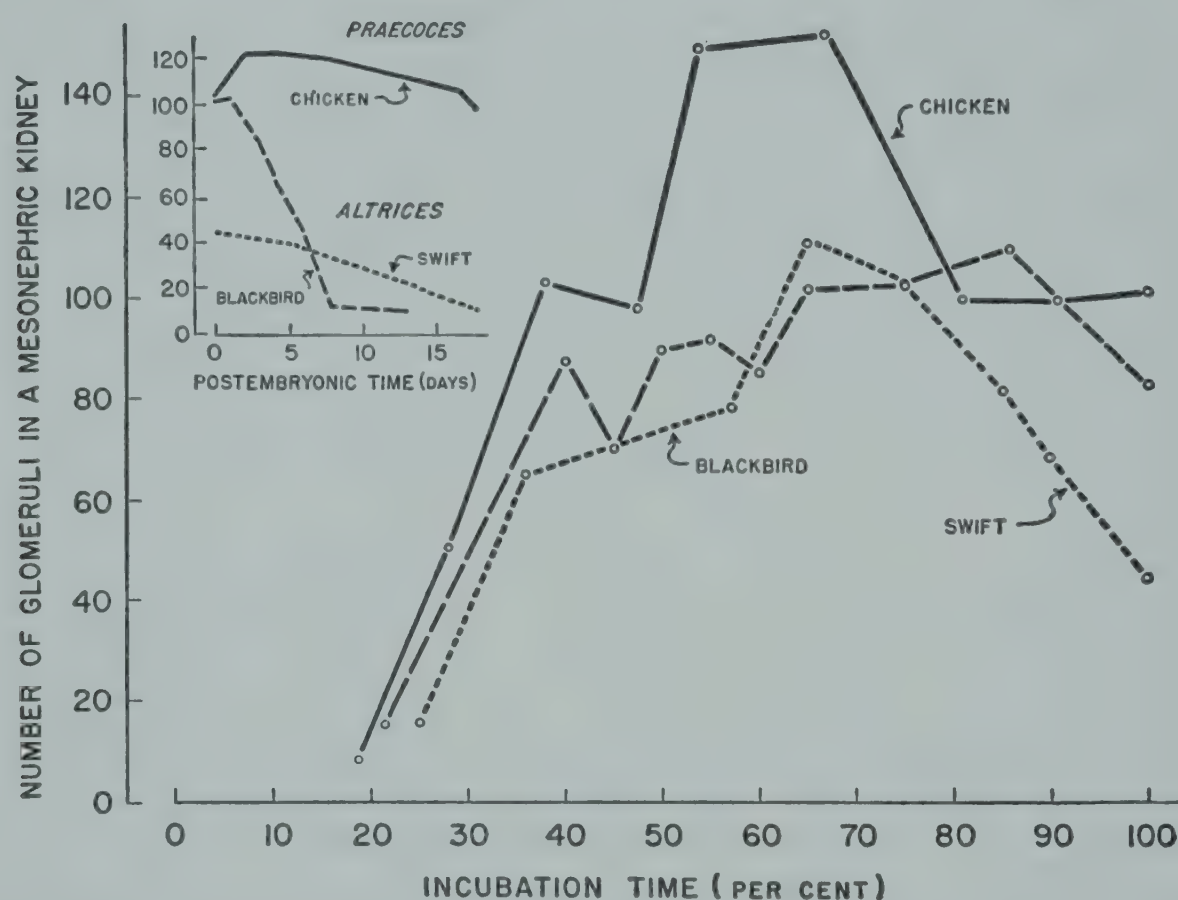


Fig. 306. The estimated number of glomeruli in one mesonephric kidney of the chicken (*Gallus gallus*), the European blackbird (*Turdus merula*), and the alpine swift (*Apus melba*); the period of maximum mesonephric function is indicated by the greatest number of glomeruli. (Modified after Stampfli, 1950.)

*Insert:* curves showing that after hatching there is a greater number of glomeruli and a slower rate of glomerular degeneration in the kidney of the precocial bird (chicken) than in that of the altricial species (swift, European blackbird).

**Experimental physiological responses.** When cut pieces of mesonephric tubules are cultured *in vitro*, they close their ends by proliferating a plug of cells and continue activity for days within the explant. Acidifying the tissue does not inhibit the excretion of phenol red or the continued distension of the proximal tubules. Lowering the temperature to 3° to 6° C., however, renders the proximal epithelium freely permeable in both directions. Observations made *in vivo* indicate the rate of activity. Eighty-five minutes after the injection of phenol red into the egg incubated 9 days, all the dye may be removed from the blood and may be present in the allantois.



Less than 5 minutes after injection, the cells of the proximal tubules become distinctly colored; distal tubules do not assume the coloration (*Chambers and Kempton, 1933*). Cysts formed from segments of proximal convoluted tubules grown *in vitro* in the presence of phenol red have been found to accumulate fluid from the surrounding medium. The fluid proved to be hypotonic, 4 to 15 per cent more dilute than that of the surrounding medium; and proteins present in the medium were absent in the cysts. Thus fluid is accumulated against the colloid osmotic pressure of the medium as well as against a positive hydrostatic pressure (*Keosian, 1938*). Adrenal cortical preparations have been administered to cultures of teased mesonephros of 9-day chick embryos. The secretory activity of the renal proximal tubules was stimulated by the presence of Kendall's whole cortical extract and by Kendall's crystalline Compound E, but was not appreciably affected by Kendall's crystalline Compound A, by Kendall's amorphous fraction, or by l-ascorbic acid (*Chambers and Cameron, 1944*).

Mesonephric tubules of 9- to 10-day chick embryos grown in tissue culture have been found to be affected by X-ray doses of 25,000 or more roentgen units. Organized proximal tubules, after a latent period which was shortened with higher doses, ceased secretion, degenerated, and became completely cytolized. Phenol red or magnesium sulfate, which stimulates secretion, increased susceptibility to injury by X-ray. Fibroblasts were inhibited from migrating out of the graft, while those already out were cytolized. Unorganized epithelial sheets of proximal tubule cells were highly resistant to X-ray (*Chambers and Cameron, 1941*).

### *Degenerative Process*

Just as tubular response to dyes is indicative of the initiation of function, it serves to indicate the cessation of mesonephric activities. Measurements also reveal the beginning of degeneration, for a decrease in number, size, and volume of glomeruli and tubules is correlated with the process. Although the rate of change varies among species, it is generally true that activity ceases near hatching time and mesonephric functions are transferred to the metanephros. The use of various chemicals can produce degenerative changes in nephric tubules.

**Beginning of degeneration.** The onset of degeneration in the chick mesonephros has been variously placed on the eighth to ninth day (*Mihalkovics, 1885*); on the thirteenth day, when ferrocyanide and phenol red are first removed (*Gersh, 1937*); on the twelfth to thirteenth day, judging by decrease in volume of glomeruli and disappearance of secretory cells from the proximal tubule (*Stampfli, 1950*); on the sixteenth day, when weight loss sets in (*Haffen, 1951a*); and on the eighteenth or nineteenth day, on the basis of dye studies (*Atwell and Hanan, 1926*). Degeneration has been noticed first on the fourteenth to fifteenth day in the swift, *Apus*



*melba*, and on the eighth to ninth day in the European blackbird, *Turdus merula* (Stampfli, 1950).

**Degenerative changes.** Degeneration is characterized by progressive disappearance of the lumen of the tubules (cf. Fig. 304-B) and the transformation and shrinkage of most of the tubules to solid cords. This is accompanied by pycnosis of nuclei, the appearance in the lumen of clusters of cells detached from the tubule epithelium, and the disappearance of the basement membrane of the tubule. Certain tubules transform to epididymal tubules, the Bowman's capsule grows toward the testes, glomeruli are surrounded by connective tissue and then degenerate, and other tubules degenerate and become connective tissue. In the main segment, pigment granules appear everywhere, and the nuclei become very darkly colored. Between the fifteenth and twenty-first day of incubation, the mesonephros loses 77 per cent of its weight, partly through water loss, partly through the loss of nitrogenous substances (Haffen, 1951b).

It can be seen from figures 7 and 8 that the rate of glomerular differentiation differs in precocial birds, like the chick, and in altricial species, such as the European blackbird (*Turdus merula*) and swift (*Apus melba*). In the former, the glomeruli occur in greater number and volume at the time of hatching, and they persist considerably longer after hatching.

Although it is commonly stated that the mesonephric bodies and ducts of the female chick degenerate shortly after hatching (Firket, 1920), Brode (1928) observed distinct remnants of right and left mesonephroi as well as of mesonephric ducts. These persisted in females until sexual maturity and were observed in hens as much as 1.5 years old. The mesonephroi are readily distinguishable from the three-lobed metanephroi and appear as small strands of tissue on the lateral walls of the postcardinal vein.

**Cessation of function.** Mesonephric function has been observed to cease in the chick on the fifteenth day (Stampfli, 1950), on the sixteenth to seventeenth day (Mihalkovics, 1885), on the eighteenth to nineteenth day (Atwell and Hanan, 1926), and at the end of incubation (Hurd, 1928). In the ouzel (*Turdus merula*) and the swift (*Apus melba*) function apparently ceases on the fifteenth and sixteenth days, respectively (Stampfli, 1950).

**Experimental degeneration.** Degeneration has been induced by various chemicals, among which are DL-alanine, anthranilic acid, 3-hydroxyanthranilic acid, and salts of tantalum, thorium, and lanthanum. When injected on the tenth or fifteenth day of incubation, these may produce massive hemorrhage and marked edema and may cause disintegration and disappearance of tubules and glomeruli. If injected at 18 days of incubation or thereafter there is no effect either on the meso- or metanephros (Lankenau, Olsen, Machlin, and Benton, 1952).



### The Metanephros or the Definitive Kidney

The metanephros, or third in the series of kidneys to be formed in the avian embryo, is the most highly developed. Its functional activity begins before that of the mesonephros ceases, but very soon it becomes the sole organ of excretion. It continues to function as such throughout the life of the individual. The basic unit is the nephron. The gross appearance of the definitive kidney at the time of hatching is a three-lobed organ on the dorsal wall of the body cavity.

#### *Formation and Development*

The definitive kidney appears in the chick on the fourth day of incubation, shortly after the Wolffian ducts have opened into the cloaca. It begins as the ureteric bud, or metanephric diverticulum, a large bud from the mesonephric duct at the convexity of its terminal bend to the cloaca. The presence of the ureteric bud induces differentiation of the secretory tubules and renal corpuscles from the nephrogenous tissue of four somites. The ureteric bud develops into the ureter and furnishes the collecting tubules of the medullary part of the kidney. Mesodermal cells surrounding the tubules form the stroma and capsule of the kidney. Development is generally similar to that of the mesonephros. The kidney is formed retroperitoneally and does not acquire nephrostomal openings into the body cavity.

***Potentialities in the early blastoderm.*** Unlike the foregoing stages in kidney development, metanephric tissue is not determined in early embryonic stages. Levels posterior to the mesonephros never differentiate in chorioallantoic grafts of primitive streak or Hensen's node levels (Willier and Rawles, 1931b). Potency is not determined until prospective metanephric material is actually incorporated in the rudiments of the organ, or until the 50- to 51-somite stage (Seevers, 1932).

***Evocation of the metanephros.*** A stimulus provided by the presence of the ureteric bud is absolutely essential to metanephros differentiation (Boyden, 1932; Seevers, 1932; Gruenwald, 1937; Kumé, 1943). When one or both Wolffian ducts are prevented from reaching the cloaca (cf. Fig. 299), the ureter on that side never forms and no metanephric tubules develop (Boyden, 1927; Gruenwald, 1941a). When the metanephric blastema remains unstimulated by its physiological inducer, it persists as a dense cell mass for a long time. Furthermore, meso- and metanephric blastemas are potentially alike, and the inducing structure alone determines whether meso- or metanephros differentiation is to occur (Gruenwald, 1942b). Nervous tissue will induce mesonephric development from metanephric blastema (Gruenwald, 1943). On the other hand, it had also been stated that there is no common primordium, and that the respective blastemas are separate (Janošik, 1905).



**Ureteric bud formation.** The ureteric bud (metanephric diverticulum) arises in the chick at the end of the fourth incubation day (Abdel-Malek, 1950) and in the duck (*Anas platyrhynchos*) at the 45-somite stage (Schreiner, 1902). This structure buds from the Wolffian duct at a point

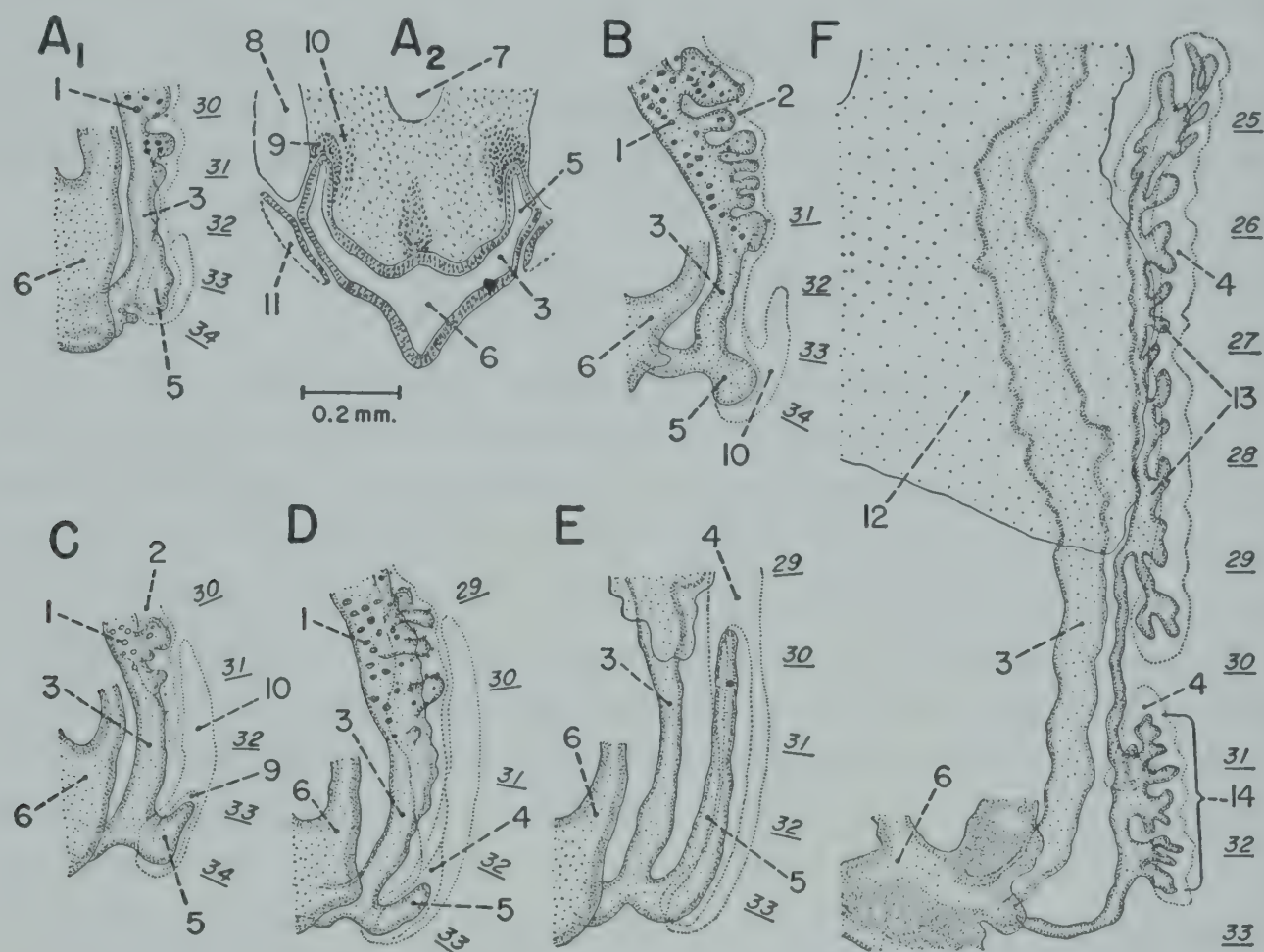


Fig. 307. The early development of the metanephros in the duck (*Anas platyrhynchos*) and chick (*Gallus gallus*) embryo. (Redrawn with modifications A<sub>1</sub>, B, C, D, E, and F, after Schreiner, 1902; A<sub>2</sub>, after Abdel-Malek, 1950.)

A<sub>1</sub>, semidiagrammatic profile view of metanephric diverticulum arising from lower end of Wolffian duct, 48-somite duck embryo ( $\times 70$ ); A<sub>2</sub>, transverse section of 4-day chick embryo showing paired metanephric diverticula budding from Wolffian ducts (in scale); B, 50-somite duck embryo ( $\times 70$ ); C, beginning of growth of metanephric diverticulum in an anterior direction, 51-somite duck embryo ( $\times 70$ ); D, continued anterior growth of metanephric diverticulum and anterior extension of metanephrogenic tissue (dotted outline) in 52-somite duck embryo ( $\times 70$ ); E, metanephric diverticulum extending from somite 34 anterior to somite 30 ( $\times 70$ ); F, profile view of primordia of metanephric tubules showing separate caudal and cranial groups, in 6-day 8-hour chick embryo ( $\times 70$ ).

1, mesonephric tubule; 2, mesonephrogenic tissue; 3, Wolffian duct; 4, metanephrogenic tissue; 5, ureteric bud (metanephric diverticulum); 6, cloaca; 7, dorsal aorta; 8, posterior cardinal vein; 9, inner zone nephrogenic tissue; 10, outer zone nephrogenic tissue; 11, coelom; 12, region of mesonephros; 13, metanephric tubules of cranial group; 14, caudal group of metanephric tubules. Underlined numbers 25 to 33 indicate somites.

just anterior to the opening of the duct into the cloaca (Fig. 307-A<sub>1</sub> and A<sub>2</sub>). The diverticulum at first resembles a knob (Fig. 307-B); then it turns anteriorly on the fifth day (Fig. 307-C). On the same day it reaches a level dorsal to the posterior part of the mesonephros. However, it remains separated from the intermediate kidney by at least two somites in which no



tubules are found (Fig. 307-D). The diverticulum then proceeds to give rise to two groups of short branches, a cranial group and a caudal group (Fig. 307-F). The latter group is formed first and, as the diverticulum progresses anteriorly, the cranial branches diverge. Eventually the anterior group is more extensive. Both groups of branches constitute the primordia of the collecting tubules. The entire lumen is lined by columnar epithelium composed of cells with large nuclei, distinct nucleoli, and basophilic cytoplasm. The ureteric branches fork dichotomously several times, forming secondary, tertiary, and additional tubules, until a complex ramified ureteric tree fills the nephrogenic area. This process occurs in allantoic cultures (Atterbury, 1923) as well as in normal development.

**Development of secreting (convoluted) tubules.** The nephrogenous tissue which forms a dense cap around the growing branches of the ureteric buds constitutes the so-called inner zone of tissue and contributes to the formation of secreting tubules (Fig. 308-A; Fig. 309-A). The outer zone of nephrogenous tissue eventually forms a dense layer providing the capsule and stroma which later surround the tubule area. As seen in allantoic cultures (Atterbury, 1923), the cells of the inner zone are often hypertrophied, having large, oval nuclei, prominent nucleoli, and deeply basophilic cytoplasm. They are closely packed and arranged radially around the tubules. The outer distal cells are loosely arranged, some are mobile, and all have large nuclei, well-defined nucleoli, and a narrow rim of basophilic cytoplasm. They are the same size as the surrounding mesenchymal cells. As soon as secondary ureteric branches are formed, the nephrogenous tissue becomes a comma-shaped mass (Fig. 308-B) and then splits into two types of masses. Part is carried forward at the growing tip of the secondary tubules, while the rest forms small dense masses of tissue that stay in close proximity to the primary and secondary ureteric branches (cf. Fig. 308-C). These islands of nephrogenous tissue are typically located in the angle between ureteric branches. From the metanephric spheres develop the secreting or uriniferous tubules. Thus they are formed entirely separately from the collecting or ureteric tubules.

The solid metanephric spheres next form vesicles preparatory to the lengthening and bending which eventually produce the typical tubular configuration (cf. Fig. 308-D and E). Development in tissue culture, as observed by Rienhoff (1922) and Atterbury (1923), is similar to the normal process. The epithelial-like cells in the solid sphere become radially arranged (Fig. 309-A). They become basophilic, and their pointed central ends begin to stain pink. These pointed ends next become flattened and push apart as an acid-staining fluid appears in the middle of the sphere (Fig. 309-B). Slightly later, a distinct lumen makes its appearance. Thus the lumen may be the mechanical result of secretory activity of the epithelial-like cells. In the chick, the lumen is established at 8 days (Rien-



hoff, 1922) and contains a fluid-like medium in which there are granules and cellular detritus (Fig. 308-F). The lumen is bordered by a high, non-ciliated, columnar epithelium. As the nephric vesicles grow in length, they bend three times, forming the characteristic S-shaped tubule which is blind

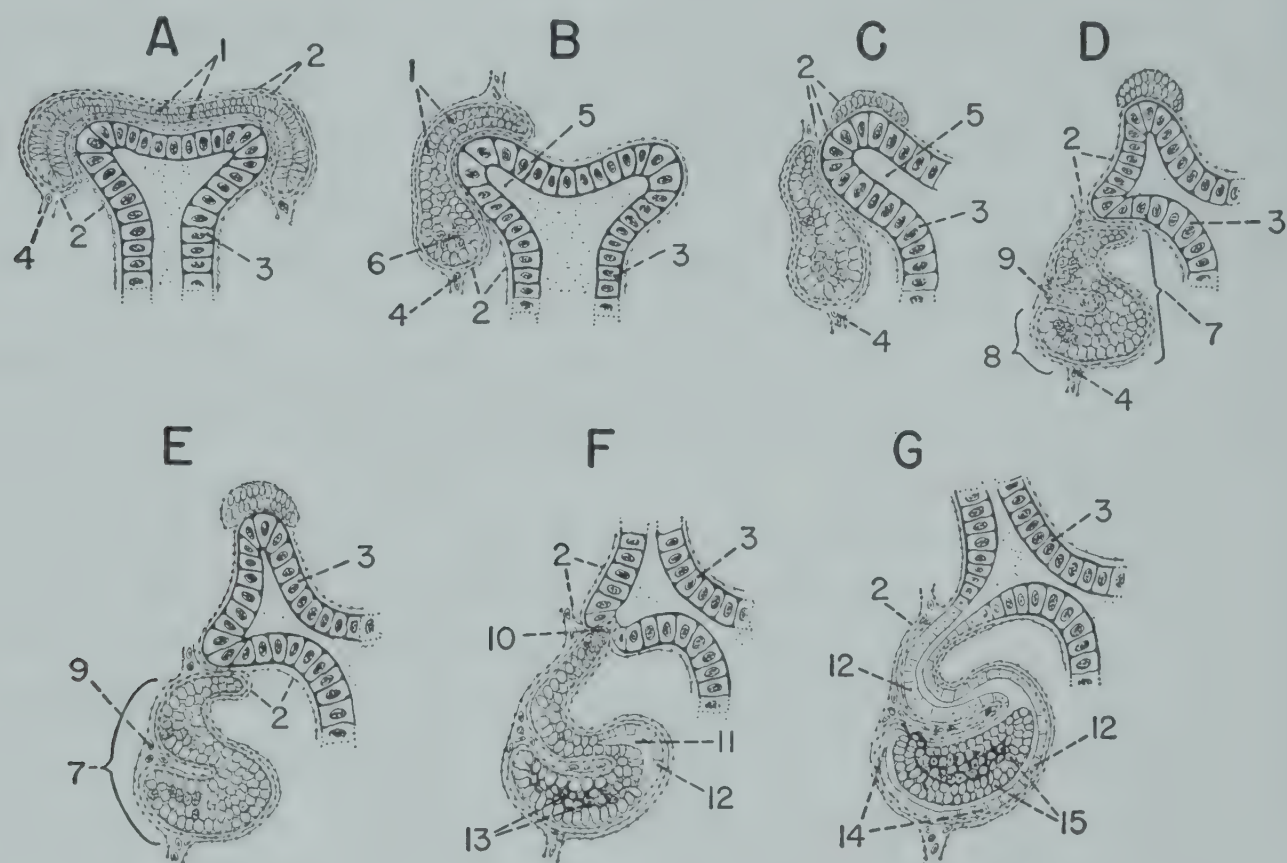


Fig. 308. Diagrammatic representation of formation of metanephric secreting tubule from metanephrogenic tissue which is in close association with collecting tubule. Successive steps in the series all occur in cultures of chick embryos of 8 to 10 days' incubation. (Redrawn and modified after Rienhoff, 1922.)

A, metanephrogenic tissue cap at growing end of collecting tubule; B, growing collecting tubule buds; C, left branch of collecting tubule slightly larger, metanephrogenic tissue at definitive position; D, secondary dichotomous branching of collecting tubule, metanephrogenic tissue forms solid, S-shaped tubule; E, glomerular end consists of spherical epithelial cells; F, S-shaped tubule becomes collecting tubule, lumen formation in larger curve, endothelial cells in glomerular region; G, lumen complete with Bowman's capsule formation. All  $\times 180$ .

1, spherical epithelial cells in cap of metanephrogenic tissue; 2, endothelial layers; 3, columnar epithelial cell of collecting tubule; 4, blood cell; 5, granule-containing lumen in left branch of collecting tubule; 6, marked cell proliferation in head of comma-shaped mass; 7, S-shaped solid tubule; 8, bulbous glomerular end; 9, blood cell in saclike protrusion of sinus; 10, point of near fusion of secreting tubule and collecting tubule; 11, beginning of lumen; 12, cuboidal epithelial cell; 13, endothelial cells (appear as dark lines); 14, Bowman's capsule; 15, spherical epithelial cells composing tuft enclosed by Bowman's capsule.

until it opens into a ureteric tubule (Fig. 308-G; Fig. 309-G and H). Rienhoff (1922) stated that the convoluted tubules develop *in situ* from a comma-shaped mass of undifferentiated tissue by mitotic growth along the length of the tubule and especially at its ends. The S-shaped tubule has an upper limb, a middle portion, and a lower limb. The Bowman's capsule



forms from the distal part of the lower limb (cf. Fig. 308-G) in much the same manner as the comparable structure in the mesonephros. Thus the convoluted tubules, the glomeruli, and the capsules of the latter develop as one unit and unite secondarily with the collecting tubules. The stroma normally give rise to sparse connective tissue cells, but in allantoic grafts (Atterbury, 1923) it is transformed into large scattered foci of granuloblastic tissue.

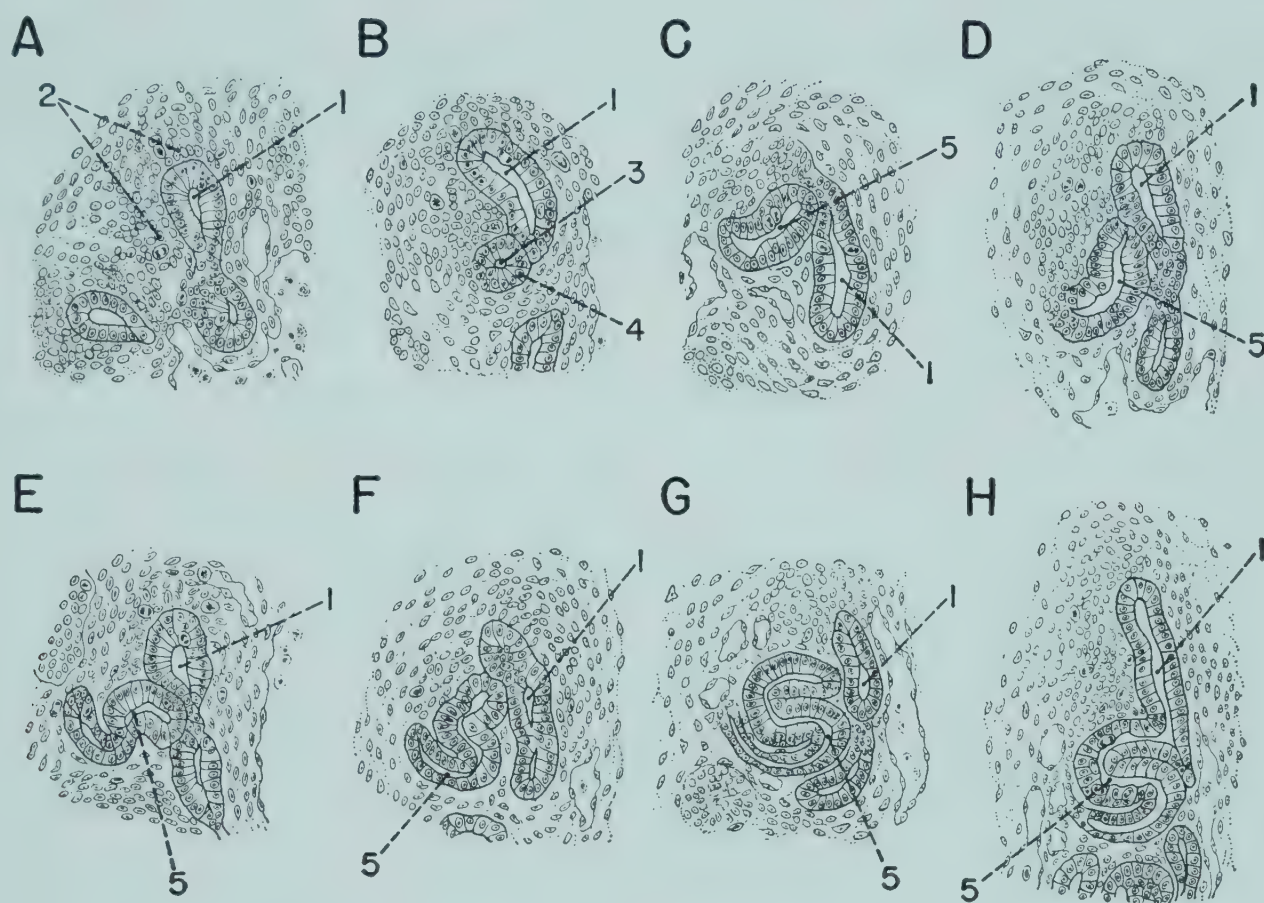


Fig. 309. Formation of the S-shaped metanephric tubule in the chick embryo. (Redrawn with modifications after Schreiner, 1902.)

A, primordium of secreting tubule represented by a solid concentration of cells forming a cap around the end of a collecting tubule; B, secreting tubule a sphere with a lumen, and located close to collecting tubule; C, elongation of secreting tubule; D, curving of secreting tubule; E and F, S-shape appearing in secreting tubule; G, development of curves of S-shaped tubule; H, fusion of secreting and collecting tubule and direct connection of their lumens. All  $\times 140$ .

1, collecting tubule branch of Wolffian duct; 2, concentration of nephrogenous cells; 3, epithelial vesicle (primordial secretory tubule); 4, columnar epithelium; 5, secretory tubule.

**Further development.** At 9 days the tubules are spread over a wide area, are undifferentiated, and are sparsely supplied with capillaries. The blood supply is sinusoidal, and circulation is by diffusion. On the next day, capillaries are present in the more developed glomerular tufts and surrounding tubules. In the S-shaped nephra, the capillaries are principally on the outer surface of the developing renal corpuscle rather than in the glomerular anlage (Rienhoff, 1922; Gersh, 1937). By the eleventh day, the blood supply has increased greatly, extending to the peripheral meta-



nephrogenic tissue and more abundantly to the tubules and glomeruli. An intricate venous capillary network is present in the medulla of each lobule. The first signs of injected ferrocyanide and phenol red appear in some of the proximal convolutions. The loops of Henle are first seen on the twelfth day; the descending limb is thin, and the oval nuclei occupy almost the entire height of the cells comprising the loops of Henle. Glomeruli are active in circulation and contain ferrocyanide, when this is administered, as do also the proximal and distal convolutions. More centrally placed nephra have a greater concentration of injected Prussian blue, especially in the loops of Henle. From the thirteenth to eighteenth days, the number of differentiated tubules increases; the blood supply is improved; and glomerular spaces, nephra lumina, and loops of Henle show greater concentrations of injected ferrocyanide. Proximal convolutions contain more injected phenol red.

*Gross Anatomy of the Metanephros*

The definitive kidney in birds is fully described by Benoit (1950). It is an elongated structure, divided transversely into three lobes, the superior one being the largest. There is a marked symmetry between the paired kidneys of birds as contrasted with asymmetry in the mammalian kidneys, where the left is usually higher than the right. In structure, the avian kidney does not show a distinct division into outer cortex and inner medulla such as we find in mammals. The division of the nephros into segments is similar in birds and mammals. In birds, however, a cubic millimeter of the cortical substance is estimated to contain 90 to 500 Malpighian corpuscles as contrasted to 4 to 15 in a similar volume of mammalian kidney. Benoit (1950) suggested that the great number of nephric units is related to the high metabolic rate in birds.

The gross measurements made by Stampfli (1950) of the metanephros in several species of birds are presented in the accompanying table. The length of the body cavity is taken from the level of the anterior appendages to the cloaca. The birds were measured 2 days before hatching.

Species of Bird Embryo	Dimensions of Metanephros		Body Cavity
	Length (mm.)	Width (mm.)	Length (mm.)
Chick ( <i>Gallus gallus</i> )	21.0	6.4	40.0
Swift ( <i>Apus melba</i> )	6.3	3.6	18.5
European blackbird ( <i>Turdus merula</i> )	7.5	2.5	17.8

*Histology of the Metanephros*

A study of the histology of the metanephros made by Tchang (1923) was mentioned by Stampfli (1950) in his comparison of the metanephros



with the mesonephros. There are only minor histological differences between the two types of avian kidney, except that the mesonephros may lack the loop of Henle.

**The glomerulus.** The glomerulus of the metanephros is similar in structure to that of the mesonephros in having capillaries greatly coiled around a compact connective tissue mass. The mass is relatively larger in the definitive kidney and the capillaries correspondingly smaller. Endothelium, basement membrane, and other glomerular structures in birds are similar to those in mammals.

**Tubule structure.** The measurements of the proximal tubule obtained by Tchang (1923) are tabulated.

Structure	Chick (microns)	Goose ( <i>Anser anser</i> ) (microns)
Diameter of proximal segment	50	46
Height of epithelium	15	12
Diameter of nucleus	4	
Height of brush border	3	

The cells of the main segment possess a round to elliptical nucleus; the cytoplasm has an upper zone near the lumen and a lower, larger zone; the mitochondria are round, irregularly arranged granules; and the brush border is homogeneous, its presence characterizing this segment.

The transition segment is comparable to the descending limb of the loop in mammals, but the epithelium is higher and is not like an endothelium. The nucleus usually lies in the center of the cell. The epithelium, although columnar in the mesonephros, is more or less cuboidal in the metanephros. The entire segment may be lacking.

The middle segment is characterized by the presence of Heidenhain's rods in the interior of the cells. The rods are seen only after fixation with formol. The average diameter of the segment is 30 microns; the height of the epithelial cells is 7 microns. Cell boundaries are indistinct, but the cells rest on a clearly defined basement membrane. The round to elliptical nuclei lie close to the lumen. This segment often connects directly to the Malpighian body.

The connecting segment is the link to the ureter. It possesses the same structure as the corresponding segment in the mesonephros. It has an average diameter of 45 microns and epithelial cells 17 microns high.

### *Metanephric Function*

In about the middle of the incubation period the definitive kidney starts to function. The onset of metanephric function can be readily determined by injecting dyes and noting the activity of tubules in excreting the colored particles.



**Beginning of functional activity.** Like the mesonephros, the metanephros is apparently ready to function without delay as soon as its nephra are formed. Half a day before the beginning of function, the glomeruli are hardly discernible, portions of the tubules are not yet distinct from the mesenchyme, and the tubules may not even have a lumen. Results of experiments with trypan blue injections indicate that metanephric function begins in the chick on the eleventh day of incubation (Atwell and Hanan, 1926; Hurd, 1928). Injected phenol red and ferrocyanide also appear first in the chick metanephros on the eleventh day (Gersh, 1937). Stampfli (1950), after measuring the degenerating mesonephros, concluded that

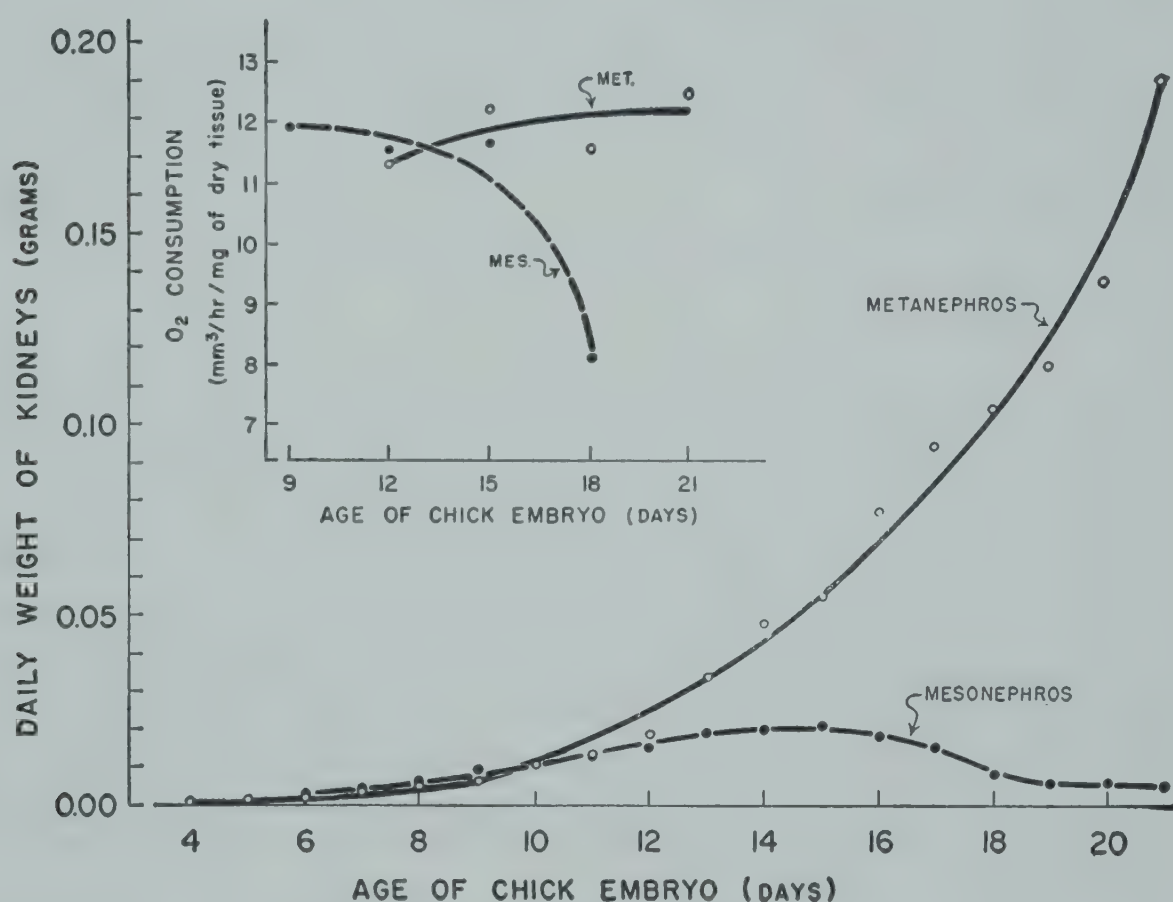


Fig. 310. Changes in weight of the mesonephros and metanephros during the embryonic life of the chick. (Plotted after the data of Kaufman, 1929b; Schmalhausen, 1927.)

*Insert:*  $O_2$  consumption of the mesonephros and metanephros of the chick, during the last two thirds of the incubation period. (After Romanoff, 1943e.)

function is transferred to the metanephros on the fifteenth day of incubation in the chick and in the swift (*Apus melba*), and on about the eleventh day in the European blackbird (*Turdus merula*). The weight of the embryonic chick metanephros increases steadily (Fig. 310) from the sixth day to hatching. The weight of the mesonephros increases but slightly up to the fifteenth day and then decreases.

**Experimental physiology.** Functional activity has largely been determined by the use of dyes. For a brief period of time the meso- and metanephros function simultaneously, and excretion is not interrupted at the time of mesonephros degeneration. Ferrocyanide appears principally in the



glomerulus of the metanephros, the lumen of the loop of Henle, and the lumen of the distal convolution; to a slight degree, it appears in the lumen of the proximal convolution and the lumina of the larger ducts. Phenol red appears chiefly in the cells of the proximal convolution (*Chambers and Kempton, 1933*) and in the lumen of the distal convolution (*Gersh, 1937*). In an investigation on chick embryos, Gersh (1937) observed that water reabsorption took place to some extent in the proximal convoluted tubule but occurred chiefly in the thin (descending) loop of Henle. This loop of Henle began to function as soon as its cells became thinner than those of the undifferentiated tubules and their round nuclei extended almost the entire height of the cell. These changes occurred after glomerular and tubular elimination were well established. Working with trypan blue, Hurd (1928) found that the dye was not picked up as intensively by the metanephros as by the mesonephros and that intracellular granules of dye were present only in cells of the proximal convoluted tubules, and more abundantly near the neck of the tubule than in the distal portion.

Grafts of duck (*Anas platyrhynchos*) metanephros grown on chick chorioallantois have been found to be active in storage of trypan blue, but less dye is stored than in the normally developing duck embryo (*Sandstrom, 1935*). A phagocytic digestive capacity has been observed in the developing chick ureteric epithelium cultured on chick allantois (*Atterbury, 1923*). This activity, not seen under conditions of normal development, appeared to be a response by the epithelial cells to the presence, in the lumen of the ureter bud, of numerous erythrocytes extravasated during extirpation.

Neither the meso- nor metanephros of amniotes acts as an organ of blood formation in either the embryo or the adult. In chick embryos, however, Danchakoff (1916) found evidence of a hemopoietic potency in the mesenchyme stroma of both types of kidney under conditions of development in culture. Hemopoiesis has been observed in transplants of duck (*Anas platyrhynchos*) and chick metanephric primordia (*Milford, 1941*). A detailed study of the development of the stroma of the metanephric anlage showed that granuloblastic tissue was formed (*Atterbury, 1923*) instead of the usual connective tissue cells. The granuloblasts were deeply basophilic, amoeboid cells, having acidophilic granules in their cytoplasm and a slightly polymorphic nucleus.

Experiments with metanephric grafts have dealt largely with heteroplastic transplants to determine species specificity. Duck (*Anas platyrhynchos*) metanephros cultured on the chorioallantois of the chick has no noticeable effect during incubation, but the host's death occurs within 48 hours after hatching. The reciprocal relation, chick-on-duck, shows a comparably high mortality rate at hatching time. Death of the host results from an apparent agglutination of the blood cells by intracellular substances



which can be released from the cells by maceration (*Sandstrom, 1940*). On the other hand, duck metanephric rudiments have been successfully transplanted to the coeloms of chick embryos and grown to hatching; apparently no species specificity existed in these cases (*Milford, 1941*). Milford suggested that the success of heteroplastic transplants is due to their ability to become adapted, as evinced by their active functioning.

## THE GENITAL SYSTEM

The genital system of birds comprises primary organs or gonads in which the germ cells develop and accessory genital organs which conduct germ cells from the body. The male gonads are paired testes while the female possesses one functional left ovary and a rudimentary right ovary. The testes consist of tubules which produce the germ cells, while the ovary has sex cords which give rise to follicles or young ova. Accessory ducts include the paired vas deferens, a derivative of the mesonephric duct in the male and the oviduct in the female. The right oviduct degenerates and the left one is functional.

The avian reproductive system, like that of other higher vertebrates, begins in the individual with the appearance of the primordial germ cells. Following this event, all later parts of gonads and accessory sexual structures are formed to function in the perpetuation of the germinal line of descent. These various parts of the genital system arise as localized thickenings or outgrowth from generalized morphological units which have been derived from one or more of the three germ layers of the embryo. The gonad primordium begins as a thickening of the coelomic wall of the urogenital ridge, a complex structural unit formed from the mesoderm through a series of morphogenetic changes. When germ cells, already formed, migrate to their definitive positions, gonad development ensues.

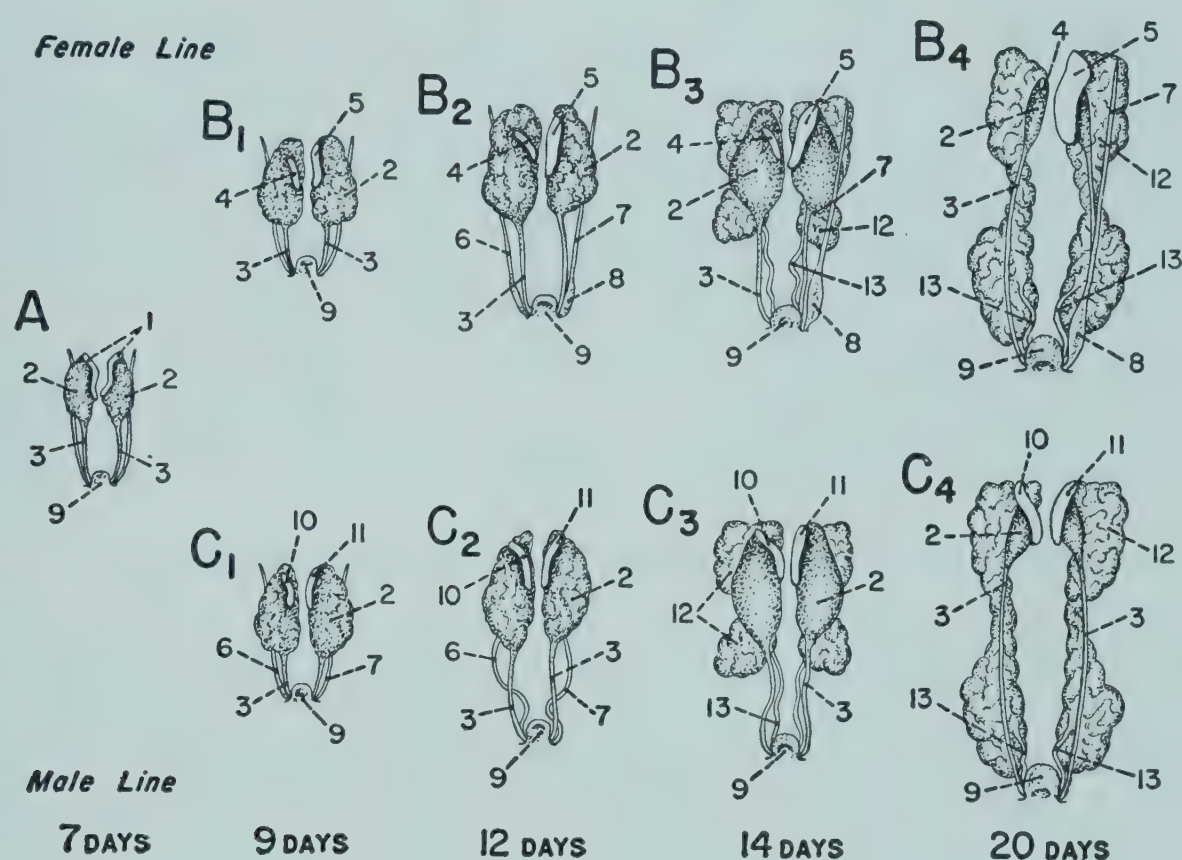
### The Early Gonad

The early gonad is located on the median surface of the mesonephros. Anteriorly, the mesonephros contributes to the ridge from which the gonad is formed. Posteriorly, the mesonephric ducts contribute to the sex ducts in the male; in the female, they are closely associated with the oviduct before becoming rudimentary.

The primordial gonad is typically composed of a medulla and a cortex which arise as successive proliferations of "sex cords" from the germinal epithelium. The cortex is potentially ovarian tissue and the medulla is typically testicular. The two components differ in intensity of hormone production. A basic asymmetry is characteristic of the development of most avian gonads (Fig. 311). Typically, the left gonad primordium possesses an incipient ovarian cortex surrounding the medulla, whereas the right



primordium consists of a medullary component and mere traces of an incipient cortex. Thus the right gonad possesses more male potentialities from the beginning, while the left gonad is potentially bisexual; and in the female, the right gonad is destined to disappear altogether. The eventual predominance of one sex or another is a matter of the degeneration of one component and the further differentiation of the other. That this is controlled by hormones, which in turn are acted upon by genes, is quite certain, for the earliest appearance of hormones has been associated with the period of sexual differentiation.



**Fig. 311.** Development of the urogenital system in the male and female chick embryo from 7 to 20 days of incubation. (Redrawn with modifications after Greenwood, 1925.)

A, sexually indifferent stage; B<sub>1</sub>–B<sub>4</sub>, stages in female development; C<sub>1</sub>–C<sub>4</sub>, stages in male development. All natural size.

1, undifferentiated gonad; 2, Wolffian body; 3, Wolffian duct; 4, right ovary; 5, left ovary; 6, right Müllerian duct; 7, left Müllerian duct; 8, shell gland; 9, cloaca; 10, right testis; 11, left testis; 12, metanephros; 13, ureter.

### *Developmental Potentialities*

Attempts have been made to ascertain when and how early in the development of the mesoderm it is possible to detect the localization of gonad-specific areas. Analysis of the histogenetic potency of isolates of early chick blastoderms, grown in chorioallantoic grafts (Willier and Rawles, 1935), shows that the urogenital complex is localized near and just posterior to the primitive node. It is not known at what time the urogenital area becomes bilateral.

The period of indifferent sexual development extends from about the



third day to about the eighth day of the chick's incubation period. In spite of the absence of morphological distinction, definite physiological or genetical sexual differences are already present during the indifferent period, as shown by grafting experiments. A graft of pre-gonad tissue, taken even before germinal epithelium can be identified in the 29- to 34-somite chick, differentiates into a gonad of a specific sex in about one fourth of all specimens (Willier, 1933). In these grafts, however, germ cells are already present. As incubation proceeds, there is a gradual increase in the developmental capacity of the gonadal area, indicating some ascending organization of that region. Later grafts, taken after visible differentiation of germinal epithelium (35- to 41-somite chicks), develop into gonads of a specific sex in about 50 per cent of all cases. Sex differences are definitely established at the genital ridge stage, for then there is almost 100 per cent development of grafts into gonads of one sex or the other (Willier, 1933). Corinaldesi (1926) pointed out that grafts of indifferent gonad tissue from a 3-day embryo will develop into a specific sex irrespective of the sex of the host. He concluded, therefore, that sex is preformed by the third day, in the chick. As shown by Willier (1925), the indifferent gonad of the 4-day embryo is specific and differentiated both in regard to sex and laterality.

#### *Influence of Related Structures on Differentiation*

In view of the essential role played by the mesonephros in metanephric and genital duct development, and considering the intimate relation between parts of the excretory and genital systems, there is some question about gonad development being dependent in any way on the presence of the mesonephros or associated excretory or genital structures.

***Primordial germ cells' influence on the gonad.*** The earliest germinal tissue, that is, the primordial germ cells, have a fairly lengthy history before their incorporation into the rudimentary gonads. The appearance of the germ cells in the germinal crescent, their inclusion in embryonic blood vessels, and subsequent migration to coelomic epithelium of the future gonad have been described in detail in Chapter 1.

It is quite certain that the germs do not influence gonad formation. In grafts of the germ cell crescent, containing primordial germ cells, the surrounding mesenchyme is never stimulated to differentiate in any way (Danchakoff, 1932b). Willier (1933) also cultured masses of germ cells in mesenchyme with no resulting gonad formation. If a blastoderm of an early somite stage is grown as a graft after the germ cell crescent has been removed, the germinal epithelium proceeds to form a sterile testis, thus indicating that origin and differentiation of the genital epithelium is independent of primordial germ cells (Willier, 1933).

***Effect of the mesonephros on the gonad.*** Gruenwald (1937) stated that the gonad is capable of completely normal development even in the



absence of all other organs of the urogenital system. Others feel that the presence of the mesonephros is necessary for normal gonad development. Willier (1937b) was able to get a certain degree of gonad development with no urogenital connection, but he concluded that the gonad rudiment does not arise entirely independently. Primordial gonads from a 3.5-day chick embryo, separated from the mesonephros, may fail to form a gonad with sex cords (Danchakoff, 1932b). From this, Danchakoff (1932b) concluded that all component parts of the gonad, namely, coelomic epithelium, ectodermal wandering cells, and mesonephros, are essential for gonad development.

### *The Gonad During the Indifferent Sexual Stage*

The first indication of the gonad is the thickened urogenital ridge containing germinal epithelium. During early genital differentiation, the ridge is composed of peritoneal cells and primordial germ cells which arrive by migration from anterior parts of the blastoderm. On about the fifth day of the chick's incubation period, the proliferation of gonadal structures begins with the appearance of rete tissue, which forms connecting cords between the nephric glomeruli and the future sex cords. The sex cords, or cords of the first proliferation, are formed as buds of the germinal epithelium and grow into the deeper lying stroma. They elongate, anastomose, and carry primordial germ cells with them. During the indifferent stage, there is no morphological distinction between the sexes. In the male, the sex cords are destined to form seminiferous tubules; in the female, they become medullary cords contributing to stroma. The second proliferation occurs after sexual differentiation.

**The urogenital ridge.** Early accounts of the ridge held that it appeared in the chick after 5 to 6 days of incubation (Bornhaupt, 1867; Mihalkovics, 1885) and after 6 to 7 days in the duck, *Anas platyrhynchos* (Mihalkovics, 1885). Swift (1915) was the first to see the ridge in the chick as early as the end of the third day. He described it as an elongated ridge covered with peritoneal epithelium. Venzke (1954a) gives the time of the appearance of the urogenital ridge on the medial surface of the Wolffian body as between 90 and 96 hours. At 3.5 days, the ridge contains the elongated cells of the germinal epithelium. Its anterior limit is at the level of the mouth of the omphalomesenteric artery. Posteriorly, it extends to various lengths, according to its stage of development. Willier (1939) claimed that the germinal epithelium is first discernible in the 38-somite chick. Essenberg and Garwaki (1938) followed the development of the germinal epithelium from the time of its differentiation in the 32-somite chick. It first becomes distinct from the coelomic epithelium covering the ventral surface of the mesonephros in a region caudal to the twentieth somite. Cellular activity begins in the coelomic angle or root of the mesentery.



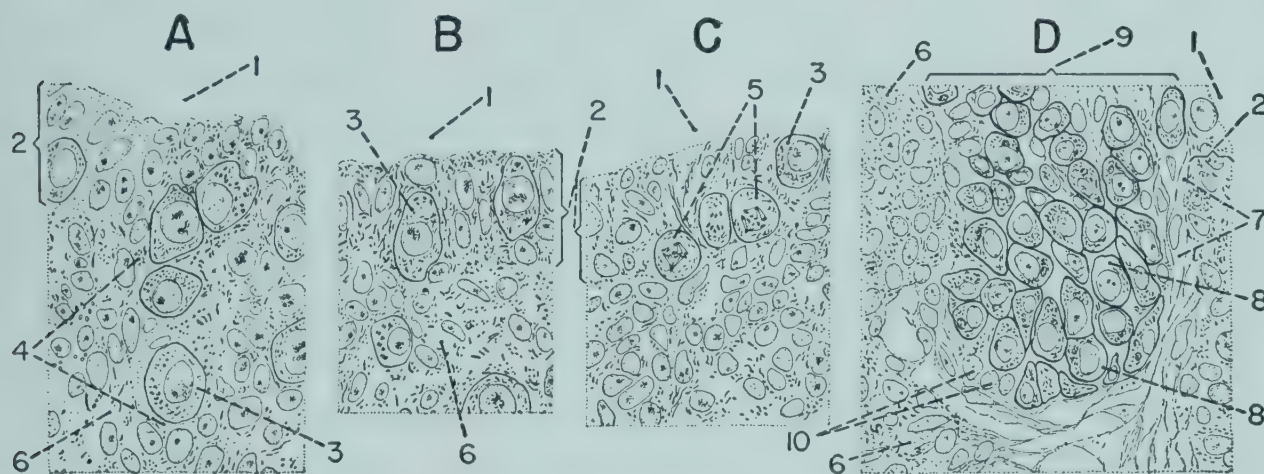
Here the germ cells settle after their migration and begin to differentiate in the single-layered peritoneal epithelium. After 83 hours of incubation, the germinal epithelium is of uniform thickness and gradually comes to consist of several cell layers, reaching its highest development during the fourth to fifth days of incubation. The germ cells at the 96-hour stage are from 8 microns to 10 microns in diameter while the peritoneal cells measure from 4 microns to 5 microns. Before 96 hours' incubation age the germ cells are rather evenly distributed between the right and left gonad. At 96 hours the left gonad contains approximately 70 per cent and the right one 30 per cent of the total germ cells counted in the gonads (Venzke, 1954a). As the germinal epithelium differentiates, the underlying mesenchyme begins to condense, preparatory to forming the stroma of the developing sex gland. The genital ridge, as described by Willier (1939), has three definite regions: the anterior, or presexual, region is the source of rete cords or cords of urogenital union; the middle part forms the sex gland of the adult and contains the sexual cords; the postsexual portion does not contribute to sex gland formation and eventually degenerates. In female birds, the left genital ridge is usually larger than the right, except in birds of prey (Stieve, 1924). By the chick's fourth day, there are two to five times as many germ cells in the left female gonad. By the fifth day the gonad anlage is greatly increased in volume because of hyperplasia of elements of the stroma (Swift, 1915) (cf. Fig. 5, Chapter 1).

**Rete cords.** In addition to the unspecialized cells and the primordial germ cells, two other epithelial structures contribute to the indifferent gonad. These are the rete cords and the sexual cords. The rete cords are the first cords to appear in both sexes. They do not produce definitive sex cells but become the rete testis in the male and remain more or less rudimentary in the female. The rete tubules basically are the connection between the true sex cords (tubuli contorti in the male) and the modified Wolffian tubules (which become vasa efferentia in the male). In the indifferent gonad the rete cords branch and anastomose, forming a network in the anterior part of the sex gland. They do not actually connect with the primary sex cords or develop lumina until the time of sexual differentiation in the male and may never progress this far in the female (Willier, 1939).

There are several views regarding the origin of the rete cords in birds. Janošik (1890) described them as being formed through the invagination of the germinal epithelium along the anterior part of the genital ridge. Hoffmann (1892) believed that they originate from the epithelium of Bowman's capsule by the production of cellular buds which grow, elongate, and extend toward the primary sex cords, eventually connecting with them. The capsular origin corresponds closely to the accepted view of rete cord formation in amphibians and reptiles. A third viewpoint has gathered



the most evidence in its support. This holds that rete cords are formed by a process of condensation of the mesenchyme which lies between glomeruli and the gonad rudiment (*Firket, 1914; Swift, 1915*). The cell composition of the early rete strands resembles the surrounding mesenchyme more than the germinal or mesonephric epithelium. The rete cords are said to arise in the chick before the ninety-fifth hour of incubation (*Firket, 1914*). The number of rete cords is sixteen according to Swift (1915), the first starting at the level of the seventeenth glomerulus.



**Fig. 312.** Stages in the development of the ovary in the chick embryo. (Redrawn with modifications after Swift, 1915.)

A, sections through ovary of 6.5-day embryo at the time of sexual differentiation, showing cords of the first proliferation (medullary cords) invaginating from the thick germinal epithelium into the medulla; B, of a 7-day embryo showing characteristic thick germinal epithelium preceding appearance of cords of second proliferation; C, of a 9-day embryo, in which primordial germ cells in germinal epithelium are dividing and clustering into small groups preparatory to invaginating into medulla as cortical cords; D, of a 14-day embryo, showing tip of cortical cord composed of oögonia and extending into medulla. All  $\times 350$ .

1, coelom; 2, germinal epithelium; 3, primordial germ cell; 4, medullary or primary sex cord; 5, primordial germ cell dividing to form oögonia; 6, medulla; 7, primary tunica albuginea; 8, oögonium; 9, cortical or secondary sex cord; 10, deep or indifferent cell or follicular cell.

**Primary sex cords.** The sexual cords of the first proliferation are the last constituents of the indifferent gonad to be formed. In the male, these become seminiferous tubules; in the female, they become medullary cords, while a second proliferation gives rise to the cortical cords.

The sexual cords arise between the middle of the fifth day and the sixth day of chick incubation (*Swift, 1916*). Cords continue to form until about the seventh day, when morphological sex differences begin to appear (*Willier, 1939*).

The cords of the first proliferation begin as budlike extensions of the germinal epithelium into the stroma (*Firket, 1914; Swift, 1916*) (Fig. 312-A). Older works give various accounts of their origin. Semon (1887) stated that sex cords are outgrowths of the capsules of the Wolffian bodies.



The observations of Swift (1916) show that growth of the bud or sex cord continues by localized multiplication of the cells of the germinal epithelium. Mitotic figures appear at the tip of the chord. In the 5.5-day female chick studied by Swift (1915), the basement membrane of the germinal epithelium is continuous around the budding cord. The cords consist of two kinds of cells; supporting (Sertoli) cells and germ cells. The supporting cells form a syncytium with nuclei which are scattered at first but later arranged next to the basement membrane with their long axes perpendicular to it. Although primordial germ cells are present in the early cords, Swift did not believe that they have a role in initiating the development of the cord. The 6.5-day chick embryo shows the beginning of separation of cords from epithelium. This process is accomplished by an ingrowth of stroma, and as a result the basement membrane is no longer continuous between the encompassing epithelium and the inwardly proliferating cords. Following separation, the sexual cords grow rapidly by increasing in diameter. They fill most of the stroma and contribute to enlargement of the gonad. They remain straight, are arranged perpendicular to the epithelium, and extend from the germinal epithelium down toward the Wolffian body. At 6.5 days the formation of cords of the first proliferation suddenly ceases, and changes then take place which permit the sex of the embryo to be determined (Swift, 1916).

### Sexual Differentiation

Sexual differentiation is generally thought to begin on about the seventh day of chick incubation (Laulanié, 1886a; Firket, 1914), although it has been also reported for the chick as starting on the fifth or sixth day (Swift, 1916; Essenberg and Garwacki, 1938) and on the eighth day (Firket, 1920). Sex is recognizable at some time between the fifth or sixth incubation day in the house sparrow (*Passer domesticus*), according to Witschi (1935a). In the Pekin duck (*Anas platyrhynchos*), males and females are distinguishable at 7.5 days of incubation (Burwell, 1931).

### *Differentiation and Development of the Testis*

In the avian adult, the testes are ellipsoid organs, both of approximately the same size, although the left may be somewhat larger. They are bean-shaped and yellowish-white in color. The seminiferous tubules are the glandular portion, and between them is mesenchyme and blood vessels. The tubules unite in seminiferous canals and leave the testes in the epididymis, which is a small projection at the medial border of each testicle. The tubules then empty into the vas deferens and thus the waste products reach the cloaca.

At the time of sexual differentiation in the male, the gonad becomes a testis, the Wolffian duct becomes the vas deferens carrying male sex



products, part of the mesonephros becomes the epididymis, and the Müllerian duct becomes rudimentary or disappears. The gonad is recognizable as a testis by the fact that it has a reduced germinal epithelium, only primary sex cords (which become seminiferous tubules), and a large amount of stroma between the sex cords, with masses of interstitial cells appearing in the stroma. The relatively similar size of right and left testes is characteristic. It is also characteristic of the developing male gonad that primordial germ cells are present in the germinal epithelium in very small numbers, or may even be absent entirely (Hoffmann, 1892; Swift, 1915).

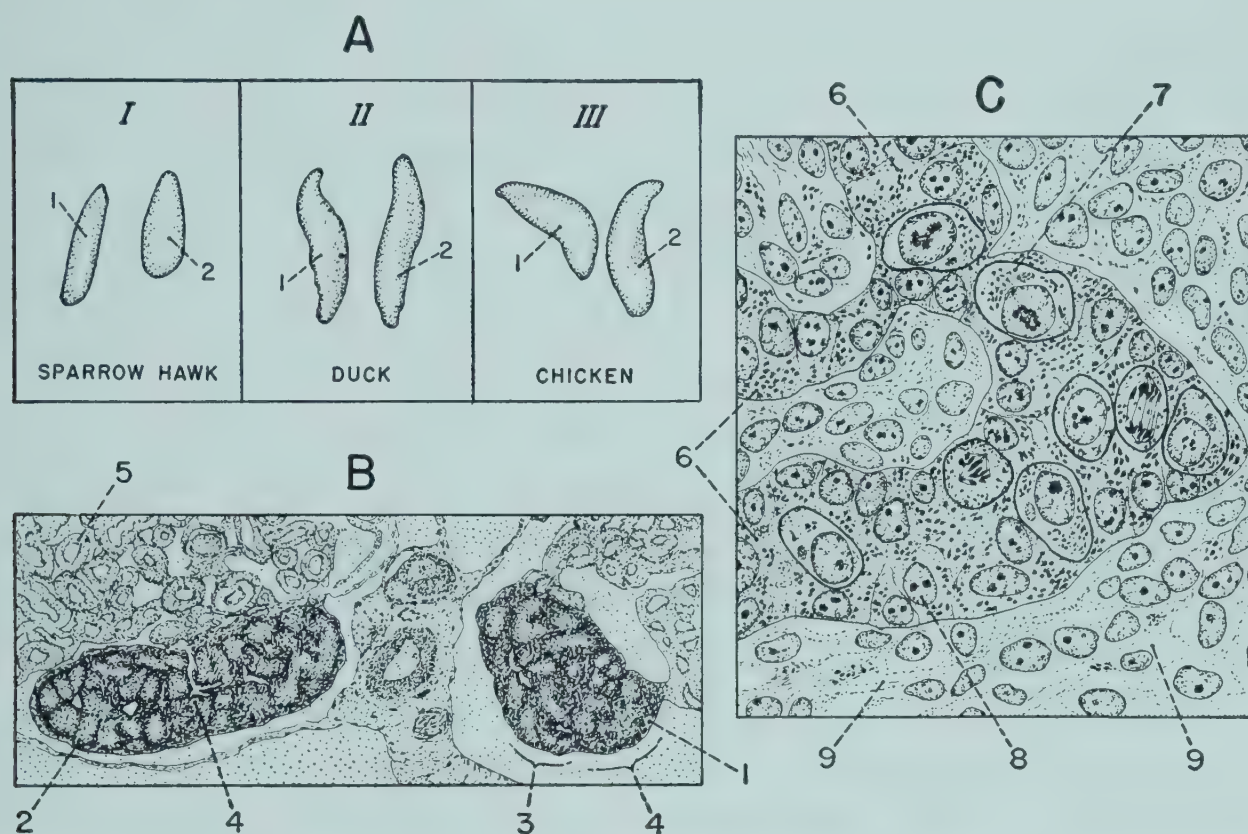


Fig. 313. Development of the testis in birds. (Redrawn with modifications A, after Kummerlowe, 1931, Lewis, 1946, and Willier and Yuh, 1928, respectively; B, after Witschi, 1935a; C, after Swift, 1916.)

A, gross aspect of both testes at the time of hatching in (I), the sparrow hawk, *Accipiter nisus*, (II), the duck, *Anas platyrhynchos*, and (III), the chicken, *Gallus gallus* ( $\times 3$ ); B, section through right and left testes and mesonephroi of house sparrow, *Passer domesticus*, incubated 8 days, the left gonad showing the cortex and medulla while the right consisting only of a medulla ( $\times 40$ ); C, section of left testis of 13-day chick embryo, showing anastomosing seminiferous cords composed of primordial germ cells and peritoneal cells, surrounded by stroma ( $\times 550$ ).

1, left testis; 2, right testis; 3, cortex; 4, medulla; 5, mesonephric tubule; 6, seminiferous cord; 7, primordial germ cell; 8, peritoneal cell; 9, stroma.

**Germinal epithelium of the testis.** In the left testis of the chick, which is more advanced in embryonic development than the right, the germinal epithelium persists beyond the sixth day (Fig. 313-B), even proliferating on the seventh and eighth days. A thickening germinal epithelium was reported by Laulanié (1886a) to persist from the seventh to the eleventh day in the left chick testis. Laulanié regarded this as homologous to the cortical layer of the ovary. A persisting epithelium has



also been noted in the duck, *Anas platyrhynchos* (Burwell, 1931; Lewis, 1946), and in the house sparrow, *Passer domesticus* (Witschi, 1935a). The character of the germinal epithelium in the male chick gonad was used as a criterion by Swift (1916) for determining sex at the earliest possible time, that is, 6.5 days. The epithelium is then a single layer thick, and the cells are cuboidal. In the left testis of the duck of 9 days' incubation, there is a well-defined germinal epithelium consisting of cells containing rounded nuclei and separated from the sexual cords by a basement membrane (Burwell, 1931). At 10 days, the germinal epithelium is of uniform thickness. It then tends to become thinner and eventually disappears anteriorly while thickening posteriorly (Lewis, 1946). Lewis believed that the thick caudal half may give rise to secondary sexual cords. On the twelfth day, she observed a scattering of multinucleate germ cells (with two to eight or more nuclei) on the margin of the gonad. These clusters of nuclei increase and may fuse to form a layer by about the sixteenth day of the duck's (*Anas platyrhynchos*) incubation. Lewis also noted that the germinal epithelium of the left testis attained its maximum thickness by the twenty-fourth day. While present, the epithelium represents a potential ovarian cortex and, with proper stimulation by female hormones, can give rise to a second series of sexual cords. In the right testis, the germinal epithelium degenerates when primary sex cords are formed. At the 9-day stage, the outer layer of cells of the duck's right testis is a discontinuous epithelium. It is much flattened, and its cells are quite unlike the rounded cells of the persisting germinal epithelium of the left testis (Lewis, 1946). In a study of the 12-day pheasant (*Phasianus colchicus*) embryo, Unger (1937) found a vestige of a cortex in the left testis, while the right testis was surrounded by the albuginea. In 12- to 17-day pheasant embryos, a very distinct columnar epithelium surrounds the outer layer of albuginea and forms the edge facing the coelom. An ovarian cortex on the left testis also persists until the time of hatching in the ring-necked dove, *Streptopelia decaocto* (Riddle and Dunham, 1942), and until well after hatching in hermaphrodite strains of pigeons, *Columba livia* (Lahr and Riddle, 1945).

**Interstitial tissue of the testis.** The connective tissue stroma is present only in small quantity up to the chick's ninth incubation day. Then rapid growth ensues all around the sex cord reticulum and distal to it. This outer part, the albuginea, separates the cortical epithelial layer and the inner seminiferous tubule mass. In the chick, the stroma has increased to large proportions by the thirteenth day and has thus contributed greatly to the enlargement of the testis. A few interstitial cells first appear on the thirteenth day. These are scattered throughout the stroma (Swift, 1916). Their origin is assumed to be the connective tissue, which in turn is derived from the mesenchyme of the mesonephros (Swift, 1916). Swift described



the interstitial cells as being of various shapes, with round, centrally placed nuclei and dense cytoplasm. They contain a large quantity of fat. In the 15- to 17-day chick embryo there is an immense amount of interstitial tissue between the sex cords. Vascularization remains moderate. After the chick hatches, the amount of interstitial tissue decreases. The interstitial cells are thought to be the source of testosterone, the male hormone, first produced in the chick on the thirteenth day (Swift, 1916). In the right testis of the duck, *Anas platyrhynchos* (Lewis, 1946), the connective tissue between the cords and the epithelium is at first loosely organized, but by the tenth day it becomes compact. It contains many flattened nuclei, which, by the twenty-fourth day, come into intimate contact with the outer epithelial layer and thus contribute to the formation of the tunica albuginea. Spaces are left between this and the testicular cords. In the adult, the tunica becomes the fibroblastic capsule encasing each testis.

**Testicular rete cords.** The rete cords are predominantly a male structure and become functional in that sex only. After sexual differentiation, they increase in size, anastomose, and concentrate in the hilum. As the cords of urogenital union, they connect the primary sex cords (seminiferous tubules) to the tubules left in the mesonephros (vasa efferentia), and these in turn continue out through the former Wolffian duct (vas deferens). When the rete cords become rete tubules by the acquisition of lumina, they give off branches, the tubuli recti (Willier, 1939). These straight tubules extend to the inner ends of the primary sex cords. The tubuli recti are considered by some authors to be outgrowths of the primary sex cords. The number of straight tubules depends roughly on the number of seminiferous tubules. In structure, the rete tubule has a central lumen and is bounded by a layer of flattened epithelial cells. The anastomosing of the tubules results in a network of cavernous spaces (Willier, 1939). In a histological study of the adult fowl testis, Gray (1937) noted a sudden transition from the pyramidal-shaped Sertoli (epithelial) cells of the seminiferous tubules to the cuboidal epithelium of the tubuli recti. The tubuli recti then empty abruptly into the rete testis, which is a mass of irregular channels immediately dorsal to the testis. The rete testis channels are lined by squamous epithelium. The rete testis empties into the efferent ducts.

**Male sexual cords (seminiferous tubules).** After the seventh day, sexual cords constitute most of the chick embryo's testis. Venzke (1954b) observed seminiferous cords at 165 hours' incubation of the chick embryo. These cords are destined to become the seminiferous tubules of the male. The cords have an average diameter of 33 microns and contain an average of 20 spermatogonia (Kumaran and Turner, 1949a). The cords radiate toward the hilum and toward the rete cords, which are pressed into the hilum. At first, the sex cords are straight, but they become wavy at the



8-day stage and are convoluted by the 11-day stage (*Essenberg and Garwacki, 1938*). The sex cords then branch and anastomose in all directions, forming a reticulum (Fig. 313-C) by the thirteenth day (*Swift, 1916*). At the 11-day stage of chick incubation the seminiferous cords have an average diameter of 25 microns (*Venzke, 1954b*). On the fourteenth day, their average diameter is 42 microns; the diameter of the spermatogonia varies considerably, but averages 4 microns (*Kumaran and Turner, 1949a*). It is generally thought that there is only one set of sex cords in the chick testis (*Swift, 1916; Willier, 1939*).

The cells of the developing sexual cords are supporting (epithelial) cells and primordial germ cells. The epithelial cells are the more numerous type, and after the chick's eleventh day their cell membranes become indistinct (*Willier, 1939*). *Swift (1916)* observed that primordial germ cells are scattered evenly throughout the cords until the 15-day stage. They move peripherally later. Not until the thirteenth day do the spermatogonia begin to be differentiated from primordial germ cells. They increase in number and differ from primordial germ cells in having mitochondria clumped in a sphere rather than scattered throughout the cytoplasm. On the day before hatching, the seminiferous cords are massive and crowd out the stroma. *Swift (1916)* and *Willier (1939)* noted that the spermatogonia within the 20-day chick's seminiferous tubules are aligned along the basement membrane with the long axis perpendicular to the latter and the mitochondrial pole directed toward it. The epithelial cells form a syncytium, and their nuclei also line up with long axes perpendicular to the basement membrane. A cytoplasmic strand extends from each nucleus to the center of the tubule where the strands coalesce. At about the time of hatching (*Swift, 1916*), or just afterward (*Hoffmann, 1892*), liquefaction in the central axis of the tubule produces a slit, and thus the lumen is formed (cf. Fig. 15, Chapter 1). According to *Essenberg and Garwacki (1938)*, the lumen is produced by the degeneration of the larger, inactive, centrally placed cells of the tubules. The cords, although forming a network, again have a fairly definite orientation, running obliquely from the germinal epithelium toward the remains of the Wolffian body (*Swift, 1916*).

In their definitive condition, the seminiferous tubules (or tubuli contorti) are lined by multilayered germinal tissue in which successive stages of spermatogenesis can be observed. From the periphery toward the lumen are found progressively more mature germ cells—spermatogonia, primary spermatocytes, secondary spermatocytes, and finally spermatids. Between the spermatogonia are supporting cells (cells of Sertoli), which are derived from peritoneal cells. The tubuli contorti are enveloped by a thin concentric layer of fibrous connective tissue. The seminiferous tubules begin blindly at the periphery of the testis.



Lewis (1946) noted two types of cords in duck (*Anas platyrhynchos*) embryos incubated 14 to 24 days, and she concluded that a second proliferation had taken place. The larger secondary cords had become incorporated into the testis and lay very close beside the primary cords. All degrees of fusion occurred between the secondary cords, but the layer as a whole remained distinctly separate from the inner layer of primary cords. A normal 24-day male duck embryo shows this sizable layer, in which the secondary cords have peritoneal nuclei densely scattered throughout the cords, rather than the arrangement of an outer layer of peritoneal cells and a medullary central layer of germ cells that is usual in primary cords. Although Lewis did not observe any direct connection between primary and secondary cords, their proximity suggests eventual connection, as in mammals. The outer layer of secondary cords becomes less prominent after 26 days and is very inconspicuous at the time of hatching.

**Gross Aspects.** The total length of the chick embryo's testis at three different developmental stages is shown in the accompanying text table.

Incubation Age	Length of Testis	Investigator
(days)	(mm.)	
8	1.625	Firket (1920)
19	2.156	Firket (1920)
21	4.650	Romanoff (1933)

The developing gonads of three species of birds were measured by Stampfli (1950), whose data are summarized in Table 15.

TABLE 15  
Dimensions of Testes in Different Species of Birds \*

Species	Incubation Age	Gonad Length	Gonad Width	Body Cavity
	(days)	(mm.)	(mm.)	(mm.)
Chick, <i>Gallus gallus</i>	5	0.8	0.1	
(Incubation period, 21 days)	8	2.0	0.5	7.5
	14	2.1	0.8	15.0
	19	2.5	1.0	40.0
Swift, <i>Apus melba</i>	5	0.8	0.1	
(Incubation period, 20 days)	8	1.0	0.2	4.5
	13	1.6	0.4	9.0
	18	1.9	0.5	18.5
European blackbird, <i>Turdus merula</i>	4	0.8	0.1	
(Incubation period, 14 days)	6	1.2	0.2	6.0
	9	1.5	0.5	10.5
	12	2.0	0.9	17.8

\* After Stampfli (1950).



In this table the length and width represent the average dimensions of left and right gonads; the length of the body cavity is measured from the level of the anterior appendages to the cloaca.

With the continued growth of the testis in length and width, the surface which comes into direct contact with the mesonephros is gradually reduced to the region of the hilum where the rete cords pass to the renal corpuscles. This reduction of surface has occurred in the 11-day chick embryo (Swift, 1916).

Fine ridges and elevations on the surface of some duck (*Anas platyrhynchos*) testes (Lewis, 1946) are the result of multiplication of tubules within the gonad. These ridges appear after about 18 days of incubation. The caudal margin of the testis may be diffuse, because of the fact that proliferating tubules may extend as finger-like processes from the surface of the gonad into the surface of the underlying mesonephros.

Testis weight at the time of hatching varies with breeds of chicken as shown in the accompanying text table. There is also considerable variation between individuals (Jaap and Thompson, 1944).

Administration of thiourea (from 2 to 10 mg.) into the albumen of the developing chick eggs at different stages of incubation resulted in some increase in weight of the testes of the embryo, proportional to the amount of drug administered (Romanoff and Laufer, 1956).

Breed of Chicken ( <i>Gallus gallus</i> )	Natal Testis Weight (mg.)	Investigator
New Hampshire	9.7	Jaap and Thompson (1944)
Barred Plymouth Rock	9.6	"
Oklabar	6.4	"
Legbar	5.3	"
White Leghorn	3.5	Latimer (1924)

**Testicular asymmetry.** The characteristic asymmetry of the genital organs, that is, the superior development of the left gonad (Fig. 314-A<sub>1</sub> and A<sub>2</sub>), is attributed by Witschi (1935a) to a primary hereditary deficiency of the right cortical inductor. In the chick (*Gallus gallus*), the English sparrow (*Passer d. domesticus*), and the red-winged blackbird (*Agelaius phoeniceus*), studied by Witschi (1935a), the primary germ cells are equally distributed on the right and left sides after 3 days' incubation. Asymmetry is first apparent on the fourth day, when the number of germ cells in the left indifferent gonad is three to ten times greater than that in the right gonad. Germ cells are present in equal numbers in right and left medullae; asymmetry is due to the far greater number in the left cortex. In the male, sexual differentiation involves further development of both medullae. By the eighth day, the few germ cells of the right cortex degenerate, but the cortex of the left male gonad is so large that it suggests a



bisexual organ. By the time of hatching, however, this cortex also degenerates (Witschi, 1935a). Hoffmann (1892) noticed a similar condition in which the left cortex is a stratified epithelium with many germ cells compared to the reduced right cortex. In all chick embryonic stages studied by Swift (1915), the right gonad was the smaller one. Mimura's (1928) study of the domestic fowl showed that the left testis was larger and heavier than the right in 94.8 per cent of embryos, 90.6 per cent of growing chicks, and 57 per cent of cocks over a year old.

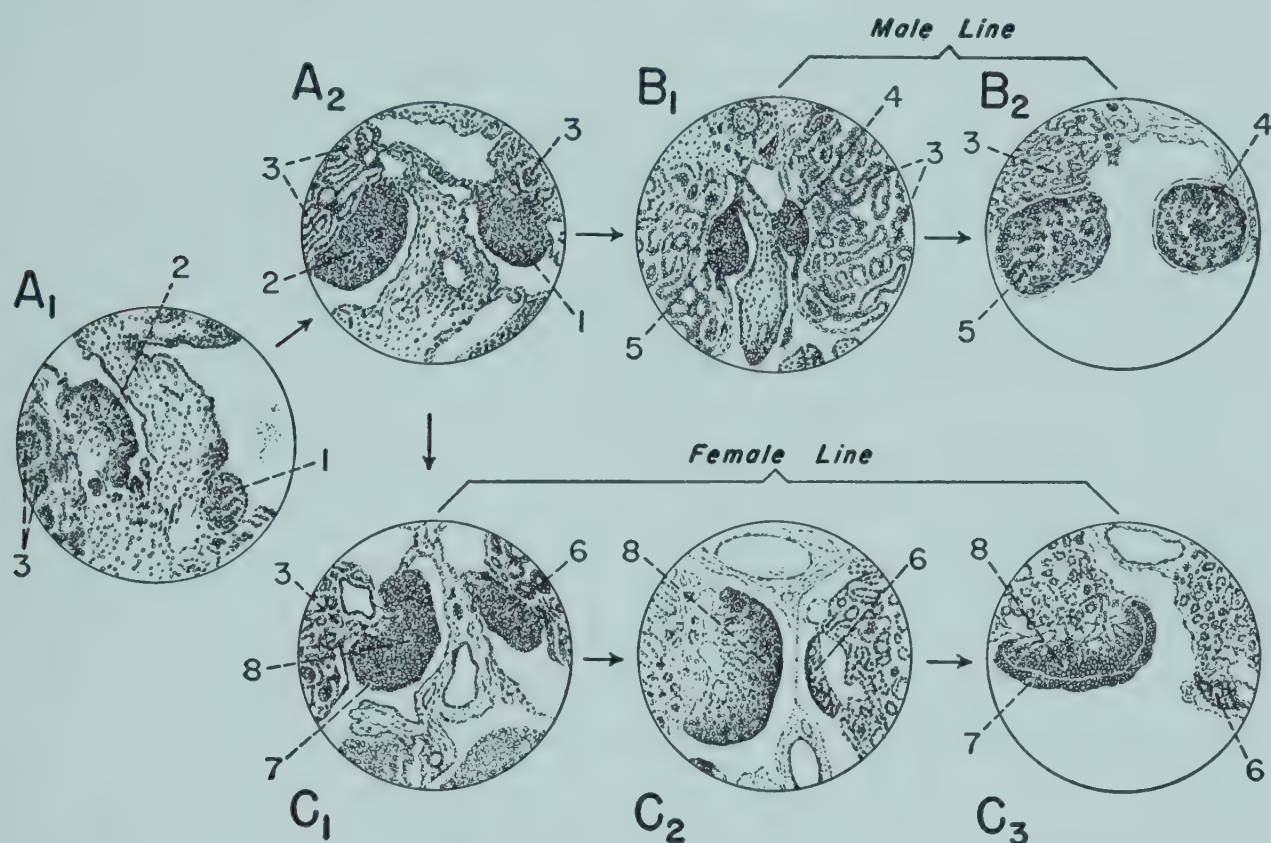


Fig. 314. The differentiation of male and female gonads in the chick embryo, in sections. (Redrawn with modifications after Zavodovsky and Zubina, 1928.)

A<sub>1</sub> and A<sub>2</sub>, indifferent stage of fourth and fifth incubation days, respectively; B<sub>1</sub> and B<sub>2</sub>, male gonads of seventh and fifteenth days; C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>, female gonads of ninth, tenth, and fifteenth days. All  $\times 10$ .

1, right gonad; 2, left gonad; 3, Wolffian body; 4, right testis; 5, left testis; 6, right ovary; 7, cortical layer; 8, medulla of left ovary.

In a study of birds of prey (Stanley and Witschi, 1940), it was shown that the right ovary is relatively very small or even absent and that the right testis is extremely small in all species in which the right ovary is markedly reduced in size.

**Natural hermaphroditism among males.** A naturally occurring hermaphroditism in very early stages of male development has been observed in certain species of birds. This intersexuality is transient. It has been noted in the chick (Laulanié, 1886b), the English sparrow, *Passer d. domesticus* (Witschi, 1935a), the European blackbird, *Turdus merula*, the ring-necked pheasant, *Phasianus colchicus* (Unger, 1936a, 1937), certain hawks (Stanley, 1937), the ring-necked dove, *Streptopelia decaocto* (Riddle and



Dunham, 1942), and the domestic pigeon, *Columba livia* (Lahr and Riddle, 1945).

### *Differentiation and Development of the Ovary*

The sexual manifestation of the female gonad differs in right and left ovaries. Whereas the right ovary undergoes degeneration, the left increases in size and complexity soon exceeding the other and the testes of the male in its proportions. Criteria for recognizing the female gonad include a thickened germinal epithelium, the proliferation of secondary (cortical) sex cords, and a thinner tunica albuginea than that of the male. In general it may be said that the cortex is more pronounced in the female as contrasted with the greater medullary development of the testis.

An injection of thiourea into the developing chick egg albumen resulted in irregular decrease in ovarian weight of the embryo, depending on the amount of thiourea used (Romanoff and Laufer, 1956).

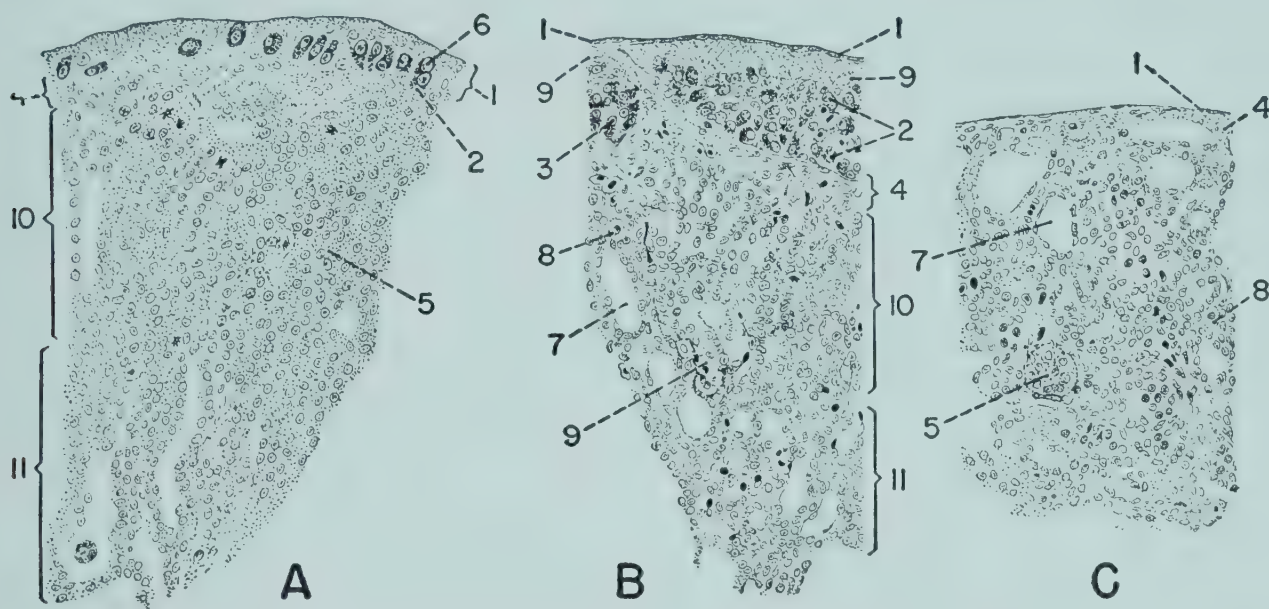
**The Ovarian Medulla.** The differentiation of the primary sex cords or cords of the first proliferation (medullary cord) is similar in both sexes. The invaginating buds of germinal epithelium consist of germinal cells and a syncytium of Sertoli cells, as described in the section dealing with the indifferent gonad. In the 7-day chick embryo, enlargement of the female gonad in all directions is attributed chiefly to an increase in medullary cords. By the 9-day stage of incubation the medulla is massive, the cords are thicker and longer, and some acquire a cavity lined by epithelium. The left ovarian medulla in the 9-day chick embryo shows two distinct layers (Fig. 315-A)—a superficial layer below the cortex and an inner reticular layer with large interstices (Brode, 1928). In the 10-day duck (*Anas platyrhynchos*) embryo, studied by Lewis (1946), the medullary tissue in the left gonad is compact, with very few primordial germ cells. During the later stages of incubation the medulla of the chick left ovary increases in size because of the formation of additional medullary cords and the distension of others. The texture of the medulla appears more porous, according to Brode (1928). He observed that the distended cords are lined by cuboidal cells and the others have squamous cells.

The medulla of the right ovary is also distended but remains thinner than that of the left. In the right medulla of the duck, *Anas platyrhynchos* (Lewis, 1946), numerous germ cells are arranged singly or in cords and are distributed both superficially and deep, so that the gonad does not appear layered into cortex and medulla. Brode (1928) noted that the right medulla is loosely constructed (cf. Fig. 315-C) and the solid and distended cords are more clearly differentiated. The 14-day duck embryo's right ovary shows spaces in the medullary tissue indicative of degeneration. Toward hatching, the chick right ovary increases slightly in size owing to the large number of distended medullary cords. The medulla of this ovary is thinner



than that of the left ovary, the former not exceeding 0.23 mm. in thickness while the latter may be up to 0.3 mm. thick (Brode, 1928). The cells of both medullae are similar, but there are more fat-laden cells in the right ovary, a fact which serves as an indication of degeneration. In the right chick medulla, solid cords of primordial germ cells are more conspicuous than ever. The medullary cords are distended and not well defined.

**Fate of the primary sex cords.** It was formerly thought that primary sex cords or medullary cords degenerated completely in the female (Firket, 1914). More recently, it has been shown that medullary cords persist after transforming into one of the following structures: distended tubules which



**Fig. 315.** Development of the chick ovary. (Redrawn with modifications after Willier, 1939.)

A, section of left ovary of 9-day chick embryo showing the invagination of the secondary sex cords from the cortex into the medulla; B, section of left ovary of 14-day chick embryo showing primary tunica albuginea and definitive ovarian albuginea; C, section of right ovary of 14-day chick embryo in which cortex is typically absent, and the medulla has a generally looser construction throughout. All  $\times 100$ .

1, germinal epithelium; 2, cortical or secondary sex cord; 3, oögonium; 4, primary tunica albuginea; 5, medullary or primary sex cord; 6, germ cell; 7, distended medullary cord; 8, medullary cord cell; 9, definitive ovarian albuginea; 10, denser superficial medulla; 11, reticular deeper medulla.

may contain germ cells; clusters or cords of clear or fat-laden cells; isolated medullary cord cells; isolated primordial germ cells; cords of primordial germ cells (Brode, 1928; Willier, 1939). With the transformation of primary sex cords, the medulla of the left ovary acquires two distinct zones (Willier, 1939). The outer zone, next to the cortex, is a compact layer, while the inner medullary zone is reticular because of distension of the medullary cords. The distension of the inner zone is the main factor in the enlargement of the left ovary.

The right ovary exhibits a similar structure, but on a small scale, and lacks a cortex. Soon after hatching, according to Brode (1928), germ cells disappear entirely from the right ovary.



**The Ovarian Cortex.** The first signs of sexual differentiation in the female are evident in the cortex of the ovary. This outer gonadal investment becomes several cell layers thick prior to its proliferation of secondary sex cords. This is only the case, however, in the left ovary, which is the definitive functional female gonad. The right ovary varies greatly among individuals and species in its degree of development, but it has relatively little cortical material and may or may not have the additional proliferation of cortical sex cords (cf. Fig. 314-C<sub>2</sub>).

**The germinal epithelium of the ovary.** The first sign of sexual differentiation in the female chick is seen after 7 days' incubation and consists of a thickening of the germinal epithelium to form several layers of columnar cells (cf. Fig. 312-B). This thickening occurs in the 8-day English sparrow, *Passer d. domesticus* (Blocker, 1933).

Thus the female gonad is already distinguishable from the male, in which the same epithelium becomes thinner and flatter and ceases to proliferate cords. In the 8-day chick embryo, primordial germ cells are evenly distributed in the germinal epithelium. At this age, according to the account of Swift (1915), the germ cells begin active division to form oögonia. The next few days see a rapid increase in the number of germ cells in the epithelium. Groups of three or four clustering oögonia form lobulations of the deep surface of the epithelium (cf. Fig. 312-C). These swellings of oögonia are the buds which form cords of the second proliferation, or cortical cords (cf. Fig. 312-D). During the 9- to 14-day period studied by Brode (1928), the germinal epithelium of the left ovary is columnar and may be one to four cells thick. From the fifteenth day to the time of hatching, the germinal epithelium in the left ovary is reduced to a single layer of cuboidal to columnar cells. The cortex is thicker because of the presence of cords of the second proliferation. The picture is similar in the Pekin duck, *Anas platyrhynchos* (Lewis, 1946). Primordial germ cells are few in the medullary portion of the left ovary, in contrast to that of the right. The cortex of the duck's left ovary becomes thicker and more compact by the twelfth to fourteenth day, and its cells are very uniform and not grouped into cords. By the twenty-sixth day, the left cortex consists entirely of differentiated cortical cells.

The right rudimentary gonad in the female 9-day duck (*Anas platyrhynchos*) embryo has a cortex one or two cell layers thick lying loosely over the medulla. According to Lewis (1946), the cortex becomes progressively thinner until, by the 14-day embryonic stage, it is no more than a series of lumps of cortical tissue attached to epithelium. This condition has also been noted in the chick (Zavadovsky and Zubina, 1928) as shown in Fig. 314-C<sub>3</sub>. Brode (1928) found a variable arrangement of cortical material in the chick's right ovary at this stage. The ovary may be covered by cuboidal to flattened epithelium, and primordial germ cells may lie among



the small, flat, epithelial cells; or the cortex may have sporadic patches of cortical tissue, in which case the germinal epithelium is columnar and several layers thick. Germ cells are as numerous in these patches as they are in the cortex of the left ovary. In the mature condition of the right ovary, cortical sex cords lead from the epithelium into the stroma. Usually no albuginea is present.

**The secondary sex cords.** Secondary sex cords are characteristic of the female gonad and thus serve as a criterion for distinguishing between the sexes (Willier, 1939). Formation of these cortical (ovigerous) cords, or cords of the second proliferation, begins by a multiplication and grouping of germ cells at the inner edge of the germinal epithelium, next to the albuginea (cf. Fig. 315-A). Buds are given off on the ninth day in the chick embryo (Swift, 1915) and on the tenth day in the duck embryo, *Anas platyrhynchos* (Lewis, 1946). On the chick's tenth day, the cords are still attached to the epithelium. The cords increase in size and number. On the fourteenth day a secondary albuginea (a connective tissue layer) separates the cords from the germinal epithelium.

Because the primordial germ cells have differentiated into oögonia by the 8-day stage, the cords consist of oögonia and epithelial cells. In the latter half of incubation, the cortical cords are surrounded by connective tissue stroma which separates the cords from each other and from the medulla. In a comprehensive study of the English sparrow (*Passer d. domesticus*), however, Blocker (1933) found a complete lack of secondary proliferation and, as a result, no cortical cords.

**The ovarian tunica albuginea.** The tunica albuginea in both sexes is a layer of loose tissue between the germinal epithelium and the inner mass of primary sex cords (Fig. 315-B). In the male, the cords of the medulla remain attached to the epithelium of the cortex until the ninth day, but in the female, they are separated from the epithelium by the intervening connective tissue layer by the seventh day (Swift, 1915, 1916). Brode (1928) noted that the primary tunica albuginea is a thin layer of connective tissue separating cortex from medulla by the ninth day. From the fourteenth day to hatching, the primary tunica albuginea becomes less conspicuous and the true ovarian albuginea consists of the connective tissue stroma, mentioned in the preceding section, which isolates the cortical cords from one another and from the medulla.

**Interstitial cells.** There is considerable difference between the sexes in the time of origin of the interstitial cells. They appear after 13 days' incubation in the male chick and some time after hatching in the female chick. Since these cells are credited with hormone production, their time of origin may have a definite significance.

Brode (1928) listed the interstitial elements of the left medulla in the chick ovary as consisting of connective tissue cells of the stroma, blood



sinusoids often filled with erythrocytes, numerous small "primitive wandering cells," and less numerous eosinophilic granular leucocytes.

*The ovarian rete cords.* Rete cords in the male chick embryo are an important link between the sex cords and efferent ducts of the kidney. The rete cords arise in a similar manner in both sexes, but the development of the rete apparatus is greatly retarded in female chick embryos. The tubules anastomose and form a network, as in the male, but lumen formation is inhibited until a month after hatching. The rete mass becomes conspicuous when the cords acquire lumina (Brode, 1928).

### Gross Anatomy of the Ovary

After 9 days' incubation, the chick's gonads appear as small white bodies lying on the ventromedial surface of the pink mesonephroi. In all embryos examined by Brode (1928), the left ovary was larger, as the accompanying data indicate.

Day of Incubation	Average Length	Average Length
	Left Ovary (mm.)	Right Ovary (mm.)
9	2.5	1.5
10	3.3	2.0
11	3.5	1.5
14	5.5	2.3

The rudimentary right ovary is barely visible after hatching, and the functional left ovary is the one usually described. In its definitive position, the left ovary is situated in the peritoneal cavity near the anterior end of the kidney. It is lobulated in appearance. Most avian ovaries have transverse folds on the dorsal surface. One particularly deep groove separates the cranial and caudal parts of the ovary, according to the observations of Koch (1927). The cranial end of the ovary is slightly bent laterally (cf. Fig. 311-B<sub>4</sub>). The absolute size of the definitive ovary varies with the season, but size relationships remain constant. The most primitive ovary observed by Koch was the type found in *Lamellirostres*. It is elongated (three times as long as wide), its cranial portion is small, and the transverse folds are poorly defined. The ovaries in more highly developed species tend to be shorter, broader, and more enlarged at the cranial end, and to have more numerous and deeper superficial folds. The outer layer or cortex produces the ovarian follicles and the cortical interstitial cells. The inner medulla is composed of medullary cords and medullary interstitial cells.

*Ovarian asymmetry.* The marked asymmetry of the female gonads is present in the embryo as well as in the adult. The right ovary owes its reduced size to the nearly complete lack of a cortex; the medulla is its principal constituent. Firket (1920) claimed that the concomitant existence of two genital glands in the female is anatomically unfavorable



when the ovary must be a large one. The compensating factor is the decreased activity of the right germinal epithelium.

The work of Witschi (1935a) shows that gonad primordia are asymmetrical from the very beginning in the chick (*Gallus gallus*), the English sparrow (*Passer d. domesticus*), and the red-winged blackbird (*Agelaius phoeniceus*). During the indifferent gonad stage, germ cells migrate from the right to the left side (Firket, 1920). Stanley and Witschi (1940) claimed that there are more germ cells on the left side at the 20- to 22-somite stage, before migration from right to left sides could occur. In a study of the definitive ovary in hawks, Stanley and Witschi (1940) observed a pronounced difference in right ovary size among subfamilies, namely, that the right ovary is progressively smaller in eagles (*Accipitrinae*), falcons (*Falconinae*), hawks (*Buteoninae*), and New World vultures (*Cathartidae*), and completely lacking in the owls (*Strigidae*). These conditions were found to exist even before the formation of gonad primordia, and therefore before a secondary ratio was established by the transfer of germ cells from the right to the left side.

Oviducts as well as ovaries exhibit asymmetry from the early stages. Secondary regression of the right oviduct begins in the chick during the eighth to ninth day of incubation (Lillie, 1919) and during the eighteenth day of incubation in the red-tailed hawk, *Buteo jamaicensis* (Stanley, 1937).

#### *Fate of the Right Ovary*

The right gonad develops in a manner similar to the left, as already indicated; but after hatching, it persists in a much reduced form. The cortical tissue may only appear as scattered clumps of cells. Medullary tissue formed the bulk of the rudimentary right ovary in 61 per cent of the right ovaries examined by Brode (1928). The rest of the right ovaries were composed of cortical tissue. Furthermore, the right ovary may lack secondary sexual cords and have only medullary cords; it is then potentially capable of transforming into a male gonad. When cortical rudiments are present, an ovotestis may develop under the proper experimental conditions. An ovotestis represents a male development of the medulla with testicular cords, accompanied by an ovarian cortex.

#### **Accessory Structures**

Accessory sexual structures are instrumental in conveying the germ cells or sex products nurtured by the gonads. The Wolffian duct of the male and the Müllerian duct or oviduct of the female are both present in both sexes during early embryonic stages. Before hatching, however, the Wolffian duct degenerates in the female, as does the oviduct in the male. The genital tubercle is likewise present in both sexes during embryogeny.



It retrogresses in the female and develops further in the male to form the penis or copulatory organ.

**Persistence of the Wolffian Duct.** In the female chicken the Wolffian duct persists as a vestigial structure well after hatching and sometimes throughout life. This is presumably the case in other birds. In the male, the Wolffian duct persists and serves as the vas deferens, carrying sperm to the cloaca.

**The Müllerian Duct.** The Müllerian duct is a derivative of the urogenital ridge. In the male, the duct becomes rudimentary and disappears; in the female, it becomes the oviduct on the left side and degenerates on the right side. In the chick, the tubal ridge, or strip of peritoneum which is the precursor of the duct, first appears at the anterior end of the mesonephros on the fourth day of incubation. At first the duct is merely a groovelike invagination of the epithelium covering the urogenital ridge. At its anterior end, the groove remains open to the ostium, which communicates with the coelomic cavity. The more caudal portions of the groove close over by the fusion of the edges on the fifth day, and the duct reaches the cloaca on the eleventh day.

The cells which contribute to the duct were once thought to be derived from the Wolffian duct, which grows in close proximity (*Balfour and Sedgwick*, 1879). The Müllerian duct is apparently dependent on the inducing action of the Wolffian duct for a growth stimulus, as already pointed out. The terminal growth of the Müllerian duct occurs in close proximity to the Wolffian duct; in fact, they share a common basal membrane (*Gruenwald*, 1952).

Development of the Müllerian duct in the male chick embryo ceases on the eighth day and is followed immediately by regression, which is completed on about the twelfth day (*Kölliker*, 1879, p. 984; *Wolff and Wolff*, 1947). *Stoll* (1944) noted growth until the eleventh day, when the ducts are 9 mm. long. They become narrower first in the median region of the canal and degeneration seems to progress toward both ends. They disappear by the twelfth day. The regression of the Müllerian duct in the male chick and duck (*Anas platyrhynchos*) embryos occurs between the ninth and thirteenth days according to *Lutz-Ostertag* (1954). In the Pekin duck (*Anas platyrhynchos*), the male Müllerian ducts reach their maximum development on the tenth day, and degeneration is apparent on the eleventh day. It begins posteriorly, proceeds anteriorly, and is completed by the eighteenth day except for the anterior tips, which may persist until the twenty-sixth day (*Lewis*, 1946). Regression between the eighth and tenth day in the chick is accomplished through rapid necroses of the epithelium. Cells become pycnotic and the lumen is filled with debris. After the tenth day, progressive absorption of the solid string takes place, and the string is merely a remnant of connective tissue after the thirteenth



day (Wolff and Ostertag, 1950). Wolff, Et. (1953) found that male hormones secreted by the testes directly determine necrosis of the Müllerian ducts, and cause their disappearance through proteolytic enzymes, whose presence was detected *in vitro* by ultra-microchemical and cytochemical methods.

In the female chick, the development of the right Müllerian duct ceases around the eighth day, and degeneration follows. The lumen is lost, and the duct becomes shorter by disappearing at its anterior end, leaving traces in the region of the cloaca. Stoll (1944) claimed that both ducts in the chick grow until the eleventh day and that the right duct begins to shorten after the twelfth day and continues to do so until the sixteenth day, after which it grows a little until hatching. Regression of the right female Müllerian duct takes place between the ninth and sixteenth days of incubation in chick and duck (*Anas platyrhynchos*) embryos (Lutz-Ostertag, 1954). This is evident from the accompanying table showing measurements of the developing Müllerian ducts in female chick embryos.

Incubation Period (days)	Length of Left Oviduct (mm.)	Length of Right Oviduct (mm.)	Ratio: Right/Left $\times$ 100 (per cent)
9	7.7	7.7	100.0
11	9.3	9.2	98.9
12	11.6	8.8	75.9
14	16.0	6.4	40.0
16	16.7	6.4	38.3
18	24.9	6.7	26.9
21	30.9	9.8	31.7
Hatched	35.5	9.3	27.8

In the Pekin duck (*Anas platyrhynchos*), both ducts are alike until, on the twelfth day, the left differentiates toward the definitive condition; on the thirteenth day, the right recedes in an anterior direction (Lewis, 1946).

The left Müllerian duct of the female undergoes further development to form the oviduct. The ostium, the glandular part of the tube, and the caudal shell gland (cf. Fig. 311-B<sub>2</sub>) become apparent by the twelfth to thirteenth day (Stoll, 1944). The lower end, although expanding to form the shell gland, does not open into the cloaca until well after hatching.

The difference in the size of the right and left ducts is considered by Gruenwald (1942a) to be a primary asymmetry. In measuring the length of the Müllerian ducts from forty-two chick embryos of both sexes ranging in age from 4 to 7 incubation days, he found the left duct to be the longer one in almost all cases. Asymmetry is apparently not due to secondary factors, such as the influence of the gonads, because it exists well before the gonads are fully differentiated.



The presence of testicular hormone seems to be directly responsible for reduction of Müllerian ducts in the male, as shown by the work of Huijbers (1951). Testicular grafts caused the typical male reduction of these ducts, whether the hosts were males or females. Furthermore, the degree of reduction was in proportion to the amount of testicular tissue grafted. These findings served as a fairly definite indication of the action of male hormone. In females, the age at which the testicular graft was planted was of the greatest importance. During the indifferent sexual

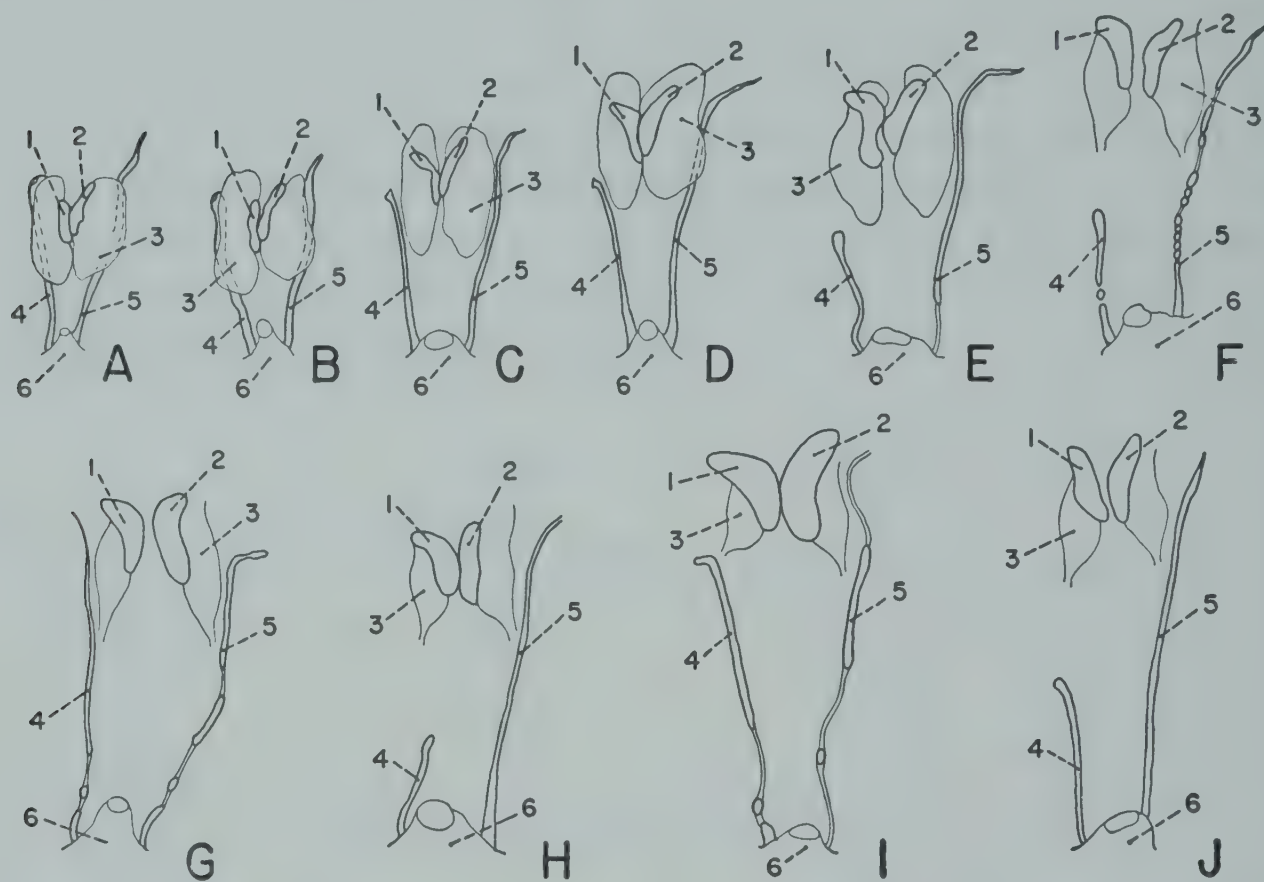


Fig. 316. Outline series showing normal development of testes and their relation to mesonephros during embryonic development, and the abnormal persistence of Müllerian ducts which has frequently been noted. (Redrawn with modifications after Snedecor, 1949.)

A, 8.75 days; B, 9.75 days; C, 10.75 days; D, 11.75 days; E, 13.75 days; F, 15.25 days; G, 16.25 days; H, 17 days; I, 17.75 days; J, 18 days. All  $\times 2$ .

1, right testis; 2, left testis; 3, mesonephros; 4, right Müllerian duct; 5, left Müllerian duct; 6, cloaca.

period, the Müllerian duct was fully responsive; but after the seventh day of incubation no reduction could be obtained. Stoll (1950) showed that androgens caused definite agenesis of Müllerian ducts if administered during the sexually indifferent stage. Estradiol, a female sex hormone, stimulated the development of Müllerian ducts in both male and female embryos (Stoll, 1944).

The effect of raising temperature was studied by Stoll (1944). A higher temperature generally retarded the regression of Müllerian ducts in incubating chicks. At  $40.5^{\circ}\text{C}$ ., 18-day males still had ducts of typical female dimensions. In females, the right duct was slower to degenerate,



and both ducts were still alike at the 18-day stage. The persistence of male ducts is interpreted as the result of insufficient androgenic hormone.

Stoll (1944) also noted the frequency of anomalies in normal development. In 506 males there were no persistent Müllerian ducts; but, out of 722 females, one embryo had oviducts on both sides, each with a shell gland, and one had no oviducts. In a study of normal development of the male genital system, Snedecor (1949) observed the abnormal persistence of Müllerian ducts (Fig. 316-A to J).

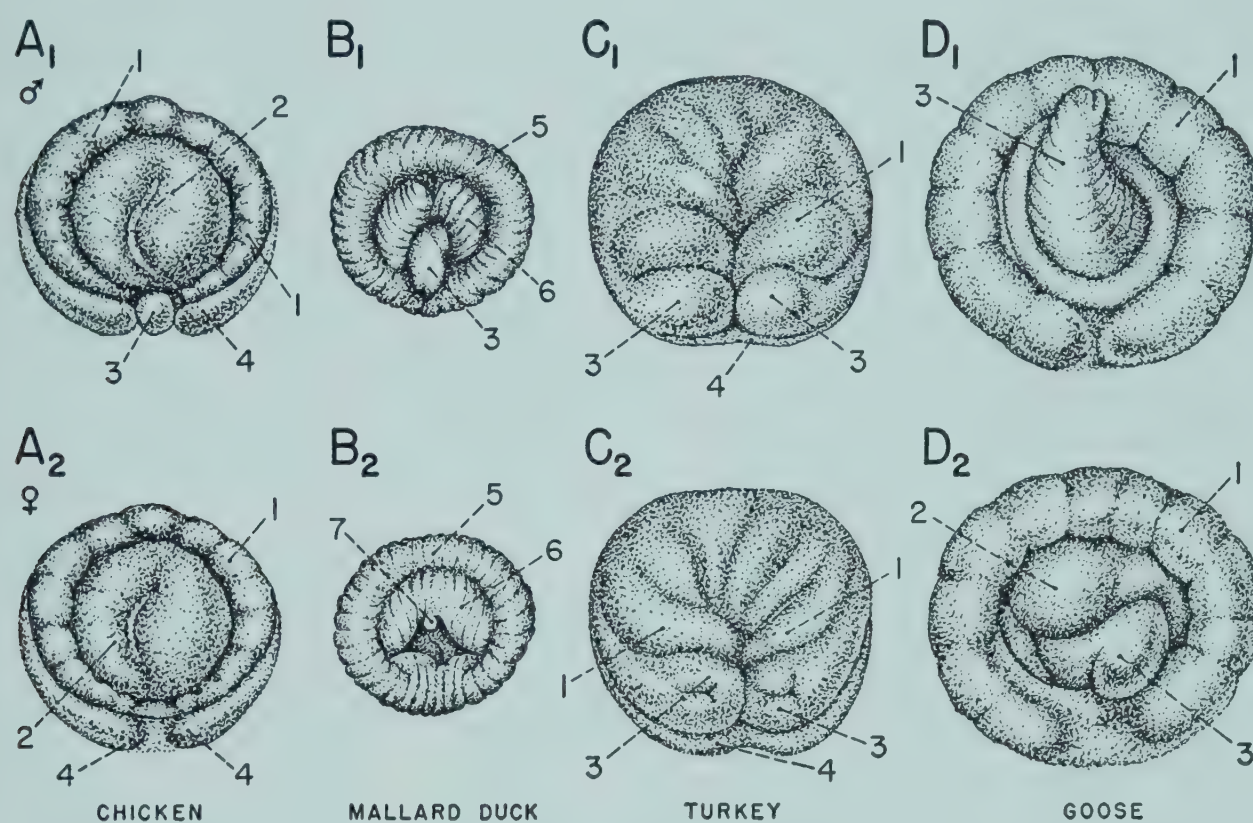


Fig. 317. A comparison of the genital eminences of both sexes in four species of birds. (Redrawn with modifications A<sub>1</sub> and A<sub>2</sub>, after Canfield, 1940; B<sub>1</sub> and B<sub>2</sub>, after Wolff, Em., 1950; C<sub>1</sub> and C<sub>2</sub>, after Canfield, 1952; D<sub>1</sub> and D<sub>2</sub>, after Canfield, 1950.)

A<sub>1</sub>, A<sub>2</sub>, male and female chicken (*Gallus gallus*) 1-day-old; B<sub>1</sub>, B<sub>2</sub>, male and female mallard duck (*Anas platyrhynchos*), 22 days' incubation; C<sub>1</sub>, C<sub>2</sub>, male and female turkey (*Meleagris gallopavo*), 1-day-old; D<sub>1</sub>, D<sub>2</sub>, male and female goose (*Anser anser*), 1-day-old. All  $\times 3.5$ .

1, cloacal fold; 2, cloaca; 3, genital eminence; 4, transverse fold; 5, external fold; 6, secondary internal fold; 7, rudimentary protuberance.

**The Genital Tubercle and the Penis.** The genital tubercle is the embryonic forerunner of the penis of adult birds. During embryonic life, the tubercle may be more or less developed. For example, only a rudiment occurs in the chick and turkey, *Meleagris gallopavo* (Canfield, 1940, 1952), while the embryo male mallard duck, *Anas platyrhynchos* (Wolff, Em. 1950) and the day-old male goose, *Anser anser* (Canfield, 1950), possess more highly developed external genitalia (Fig. 317). In certain strains of chickens the genital tubercle of the embryo male is noticeably better developed than the corresponding structure in the female, and it can be used to distinguish the sexes at an early stage.



In the pigeon (*Columba livia*), the anlage of the phallus can be identified only in its early embryonic stages. After 7 or 8 days of incubation, it appears as a relatively large raised body but quickly regresses and has almost completely disappeared in both sexes at the time of hatching (Hasimoto, 1931).

An extensive study of the penis of the male mallard (*Anas platyrhynchos*) was made by Wolff, Em. (1950). The definitive structure in the mallard is a long, conical, helicoidal organ, spiralled three times (Fig.

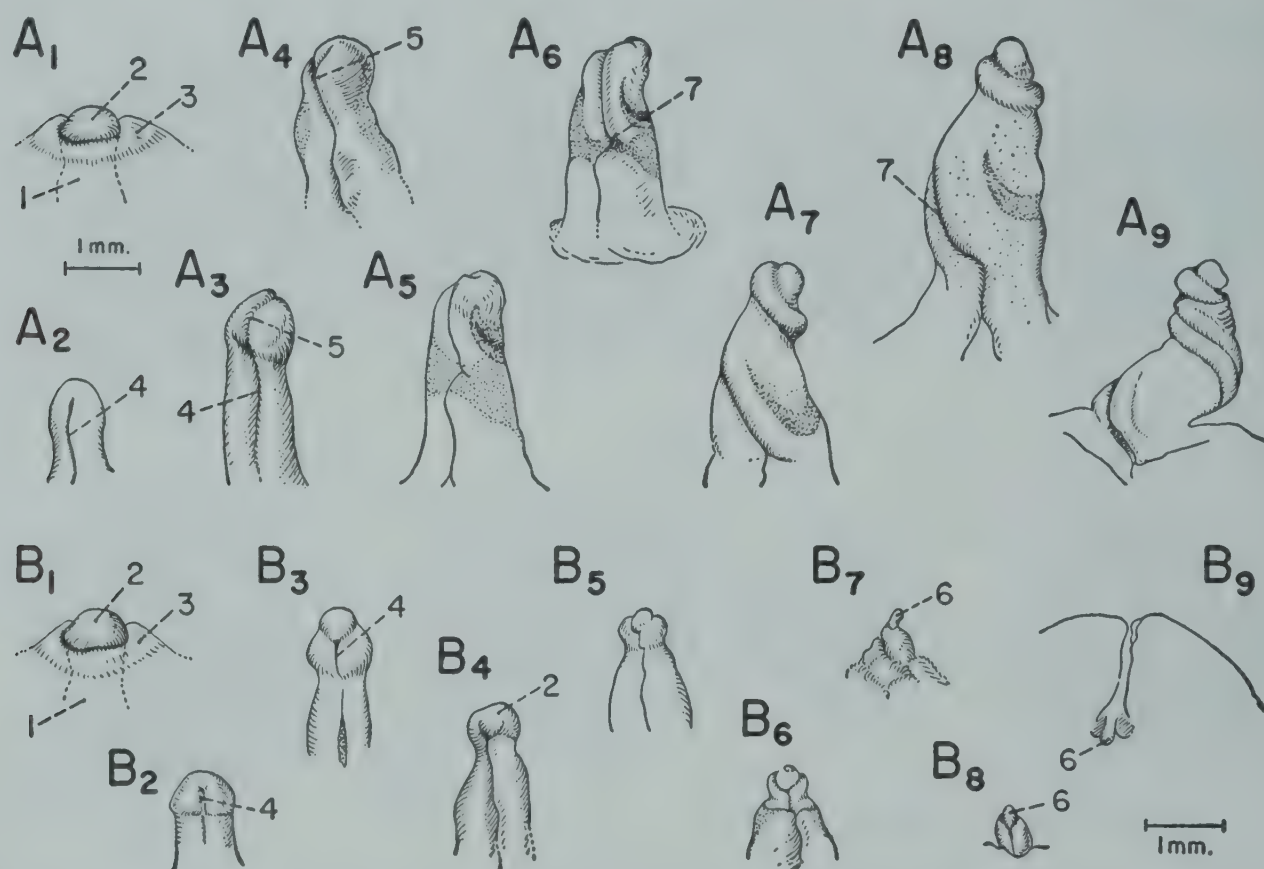


Fig. 318. The normal development of the genital tubercle in the male and female mallard duck, *Anas platyrhynchos*. (Redrawn with modifications after Wolff, Em., 1950.)

A series, male; B series, female.

A<sub>1</sub>, 9 days sexually indifferent; A<sub>2</sub>, 10 days; A<sub>3</sub>, 12 days; A<sub>4</sub>, 13 days; A<sub>5</sub>, 15 days; A<sub>6</sub>, 16 days; A<sub>7</sub>, 20 days; A<sub>8</sub>, 24 days; A<sub>9</sub>, 29 days; B<sub>1</sub>, 9 days, sexually indifferent; B<sub>2</sub>, 10 days; B<sub>3</sub>, 12 days; B<sub>4</sub>, 13 days; B<sub>5</sub>, 15 days; B<sub>6</sub>, 16 days; B<sub>7</sub>, 20 days; B<sub>8</sub>, 24 days; B<sub>9</sub>, 29 days. All  $\times 15$ .

1, base of genital swelling; 2, tip of tubercle; 3, surrounding ectoderm; 4, slitlike beginning of seminal groove; 5, dextral torsion beginning the helicoidal spiral of seminal groove; 6, rudiment of tubercle; 7, seminal groove.

318-A<sub>9</sub>). It is located at the cephalad margin of the cloacal aperture. A seminal groove follows the spiral curve throughout the entire length. At the base of the adult mallard organ there are two longitudinal swellings, of which the left is the larger. These are lymph concentrations which function in erection. The male structure is about 6 cm. long. The female duck has a clitoris about 0.8 mm. long and 0.4 mm. in diameter.

During its embryonic development, the tubercle appears in males and females at the time of sexual differentiation, which is the eighth day in



the mallard duck, *Anas platyrhynchos* (Wolff, Em. 1950), and the sixth day in the chick (MacDonald and Taylor, 1933). It originates as a small papilla on the oral lip of the proctodaeum and is identical in both sexes. During the ninth day of mallard incubation a small peduncle raises the genital tubercle above its anal base (Fig. 318-A<sub>1</sub> and B<sub>1</sub>), and on the following day a longitudinal ridge traverses the caudal side of the organ. By the eleventh day the epithelium of the ridge has sunk along its length to form a groove. The edges come together, and a cylindrical lumen is formed by the fourteenth day. In both sexes, the canal ends as a blind sac. Thus an internal blind canal, corresponding to the external seminal groove, extends along the length of the tubercle. On about the twelfth day, the distal end of the male tubercle in the mallard undergoes torsion toward the right (Fig. 318-A<sub>3</sub>), thus initiating the helicoidal spiralling of the seminal groove and tubercle. The phallic knob also begins sexual differentiation on the twelfth day in the chick (MacDonald and Taylor, 1933). Torsion is more pronounced by the thirteenth day (Fig. 318-A<sub>4</sub>) and sexual dimorphism is then unmistakable, although the size is the same in both sexes at this time. There are two complete spiral turns by the twentieth day and, by the time of hatching, almost three turns in the male. Spiralling may be completed shortly after hatching. By the eighteenth day, there is formed a circular ridge at the base of the tubercle and then a secondary concentric swelling, both of which surround the penis. At the end of incubation the inner ridge covers the penis and conceals it from external view.

On the twentieth day, the female tubercle (Fig. 318-B<sub>7</sub>) is not as well developed as that of the male. It is symmetrical, having undergone no torsion. There are two prominent swellings at either side of the small median protuberance and these more or less border the small triangular opening of the canal. An outer rim surrounds the entire structure. This structure in the female embryo grows only from the eighth to the twelfth day. By the nineteenth day it has rapidly decreased in size, and on the twenty-fourth day it is only 0.3 mm. long and 0.2 mm. in diameter (Wolff, Em., 1950). In the chick it is a flattened fold 0.55 mm. in diameter at hatching (Hsimoto, 1930). In the duck (*Anas platyrhynchos*) it is comparable to a clitoris and persists as such in the adult.

Experimental modifications of the penis are mentioned in the section dealing with hormones. As expected, the female hormone represses normal development of the penis in the male, while male hormone in either sex promotes a typical male penis to develop regardless of the sex of the host.

### Experimental Modifications of Sex

The experimental modification of sex generally deals with the production of intersexes by means of sex reversal. Various agents have been em-



ployed to this end. Generally speaking, the action of male hormones is to produce a masculinization of females, while female hormones are effective in feminizing males. This field of experimentation has been explored in detail, and a variety of sex hormone substances have been studied for their effect on both sexes. Sexual inversion can also be achieved by the use of X-irradiation, through the action of chemical substances, or by grafting and other surgical means. Intersexuality can exist in all degrees. In females it has been classified by Wolff (1935) as ranging from regression of the distal end of the oviduct or complete absence of the oviduct to transformation of the left and right gonads to an ovotestis and a hypertrophied, testis-like structure, respectively. Wolff and Ginglinger (1935*b*) described various degrees of intersexuality in males. The partial condition may be represented by the presence of a left ovotestis. In cases of total intersexuality, the left gonad is an almost perfect ovary, the right gonad is vestigial, the left Müllerian duct is the size of a normal oviduct but possesses a less dilated shell gland, and the right Müllerian duct consists only of its cloacal end.

### *Role of Hormones*

The normal action of sex hormones is generally to "catalyze" the development of gonads and accessory sexual structures. During the earliest stages of gonad differentiation, when the urogenital ridge is formed and the gonadal primordia are established, there is no evidence that hormones participate.

The actual time when hormones are first released into the blood stream of normal embryos is unknown. It is generally believed (Willier, 1952) that they must be present in the circulation of the chick by the tenth day, because they probably initiate regression of the oviduct at that time.

The probability that hormones are released into and distributed by the blood stream seems almost incontestable since distant structures like the genital tubercle and syrinx are affected by them. Castration experiments support this conclusion, for, in the absence of gonads, accessory structures fail to differentiate into well-defined, sexually distinctive organs. Instead they retain a neutral or asexual appearance.

There is evidently a diffusion gradient in hormone distribution, because grafts have the greatest effect on host gonads or gonoducts when placed close to them. It has been suggested that hormone dispersal is by gradual diffusion through the tissues rather than by the blood stream (Wolff and Wolff, 1951*b*).

Hormonal experiments are possible because of the fact that crystallized and synthesized sex hormones have the ability to produce effects essentially similar to gonad secretions.

To test the hormonal potency of a substance whose composition is



unknown, its effects are compared with those produced by a given quantity of a known hormone. The units are based on the minimal amount which will evoke some particular immediate response in a single organ. In one case, for example, the International Unit is defined as one tenth of a microgram of crystalline estrone. The International Unit Equivalent is defined as that amount of material which in its bioassay produces an effect equivalent to that of one tenth of a microgram of crystalline estrone. The rat and mouse are generally used in biological standardization of estrogens. The ratio of rat unit to mouse unit varies considerably depending on method of assay and nature of estrogen.

### *Influence of Male Hormones*

As one might expect, the general effect of male hormones is a masculinizing one. However, exceptions to this principle occur in cases where females receive male hormone treatment, or even in the case of potentially female structures in the male embryo. The naturally occurring male hormone is testosterone. Experimentally, the use of testosterone propionate has been widespread. Work with androsterone, dehydroandrosterone, androstenedione, and others, is summarized in Table 16, which shows the effects of hormone preparations.

**Effect on Genetic Males.** Male hormone substances do not act as an extramasculinizing agent when administered to males. This is surprising when one considers that female hormones produce hyperfeminization in females. Paradoxically enough, male hormones administered to males have a somewhat feminizing effect (Fig. 319-A<sub>1</sub> and A<sub>2</sub>). A series of substances having the same qualitative effect as androsterone is listed in Table 16 showing hormonal effects. The structural formulae for many of the hormonal substances are given by Wolff, Strudel, and Wolff (1948).

**Effect on gonads.** Androsterone furnishes an example of the feminizing effect in males. Injections of this natural product produce ovarian cortex development (Fig. 320-A<sub>1</sub> and A<sub>2</sub>) in the left testis and partial regression of medullary cords, thus rendering the individual an intersex. The right testis may be hypertrophied.

**Effect on accessory structures.** Male recipients of androsterone generally undergo abnormal development of the Müllerian canal (Wolff, 1937b; Wolff, Strudel, and Wolff, 1948) (Fig. 320-A<sub>2</sub>). Willier (1937a) noted similar marked feminizing effects of androsterone and dehydroandrosterone.

**Effect on Genetic Females.** The treatment of genetic females with male hormones produces a characteristic masculinization which usually varies in degree according to the dosage and the specific hormone administered. In general, the quantities of male hormone required to check female development are large when compared with the smaller dosages of female hormone that can inhibit male differentiation. The general picture



TABLE 16

Effect of Male Sex Hormones on Avian Embryonic Sex Organs and Accessory Sexual Structures

Legend: B.U. — bird units * F — female f — feminizing y — gamma * 1 bird unit = 1.5 international units									
Hormonal Substance	Dosage	Day of Treatment	Day of Examination	Species	Sex	Effect			Investigator
						Gonad		Duct	
						Medulla	Cortex		
Androstenedione	0.02-10 mg.			Chick <sup>1</sup>	M F		general f general m hyper.		Willier, Gallagher, and Koch (1936)
Androsterone	0.5-2.0 mg.		15	Chick	M	m	f		Wolff (1936b); Wolff, Strudel, and Wolff (1948)
“	0.02-2.0 mg.	2,3	16,18	Chick	M	0	f left	m	
“					F	0	0	m	Willier (1937a)
Androstane-dione	1.5 mg.			Chick	M,F			m	Gaarenstroom (1939)
				Chick	M	m	f	f	Wolff, Strudel, and Wolff (1948)
Androstene-diol				Chick	M	m	f	f	Wolff (1948)
Androstene-dione				Chick	M	m	f	f	Wolff (1948)
Methyl 17 androstanol				Chick	M	m	f	f	Wolff (1948)



Trans-androstane dione			Chick	M	m	f	t	Wolff (1948)
Trans-androsterone			Chick	M	m	f	f	Wolff, Strudel, and Wolff (1948)
Trans-dehydroandrosterone			Chick	M	m	f	f	Wolff (1948)
Dehydroandrosterone	0.02-2.0 mg.	2,3	Chick	M F		general f general m		Willier (1937a)
	1.5 mg.		Chick	M F		hyper. hyper.		Oordt and Rinkel (1940)
Male hormone from bull testis	10 to 200 B.U.		Chick	M F	0 0	may hyper. may hyper.		Willier, Gallagher, and Koch (1935b)
Male hormone from male human urine	7.9 to 150 B.U.		Chick	M F		f hyper. f hyper.	m m	Willier, Gallagher, and Koch (1935a)
Testosterone propionate	0.15-0.25 mg.		Chick	F	0		m	Danchakoff (1937a)
"	0.02-2.0 mg.		Chick	M F	0 m			Willier (1937a)
"	1.25 mg.		Duck <sup>2</sup>	M F	0 0		0 0	Lewis and Domm (1946)
"	0.18-3.25 mg.	2,6	Chick	M F			agenesis agenesis	Stoll (1948)
"	500 y 1250 to 5000 mg.	3 2	Chick Turkey <sup>3</sup>	M F	0			Snedecor (1949) Jaap, Ingram, and Godfrey (1951)

<sup>1</sup> *Gallus gallus*; <sup>2</sup> *Anas platyrhynchos*; <sup>3</sup> *Meleagris gallopavo*.



of masculinization produced by androgens usually does not include the repression of ovarian cortex development. In other words, the impetus for secondary proliferation seems to occur regardless of the interference of male hormone (Wolff, 1937b).

**Effect on gonads.** Androgens do act on the potentially testicular tissue, the ovarian medulla. The normal regression of the ovarian medulla is prevented by androgens. Moreover, with sufficient dosages, the medulla can be induced to proliferate testicular cords. Danchakoff (1937b) con-

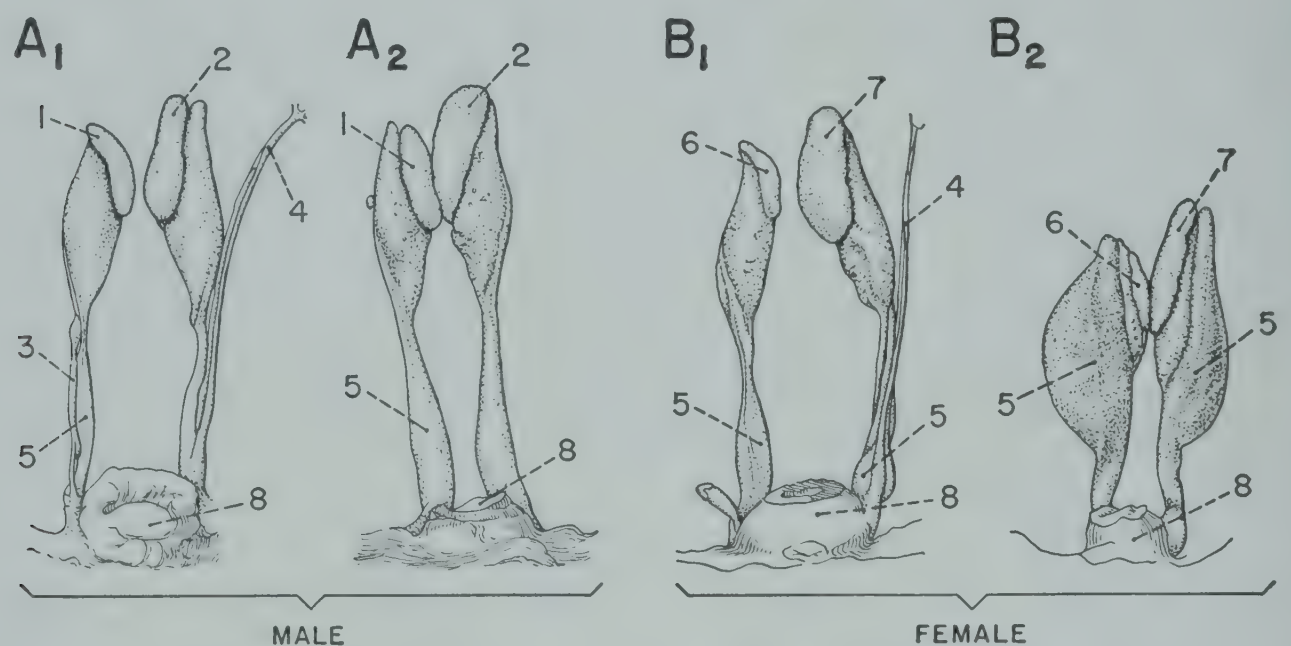


Fig. 319. The effect of male sex hormone in male urine extract and its masculinizing effect on genetically female reproductive systems and its feminizing effect on the genetically male reproductive system in the chick embryo. (Redrawn with modifications after Willier, Gallagher, and Koch, 1937.)

A<sub>1</sub>, genetic male which received 10 S.B.U. (sparrow beak unit) male sex hormone, posterior half of left gonad is an ovotestis, right is a testis, Wolffian ducts hypertrophy, and oviducts persist; A<sub>2</sub>, same treatment but entire left gonad is an ovotestis and oviducts are missing; B<sub>1</sub>, genetic female after 9.6 S.B.U. male hormone and 6.3 I.U. (international unit) female sex hormone in which only Wolffian ducts are hypertrophied; B<sub>2</sub>, same treatment but Wolffian ducts are extremely hypertrophied and oviducts entirely absent. All  $\times 2$ .

1, right testis; 2, left ovotestis; 3, right oviduct; 4, left oviduct; 5, Wolffian duct; 6, normal right ovary; 7, normal left ovary; 8, cloaca.

cluded that female embryos cannot be completely converted to males by the administration of testosterone, although various effects (such as increase of size of non-feathered areas of the head) can be produced.

**Effect on accessory sex structures.** Androgens have a definite effect on Müllerian ducts. The normal tendency for the left duct to persist and become an oviduct is thwarted by male hormone, and the duct may regress, except for the ostium (Wolff, 1937b). Although Wolffian ducts are normally very reduced in females, androgens, if present, can cause their hypertrophy (cf. Fig. 319-B<sub>1</sub> and B<sub>2</sub>; Fig. 320-B<sub>1</sub> and B<sub>2</sub>).

One of the secondary sex characteristics in birds is the marked difference



between the male and female syrinx. The distinction occurs very soon after the differentiation of the gonads. Experiments have shown that the male syrinx is the primordial one and that female hormone inhibits its development (Wolff, 1952). Male hormone can only feebly reverse the naturally occurring inhibition in females. This has been demonstrated by Wolff (1949a), who used testosterone propionate or estradiol benzoate in duck (*Anas platyrhynchos*) eggs, as well as castration by X-irradiation of gonads.

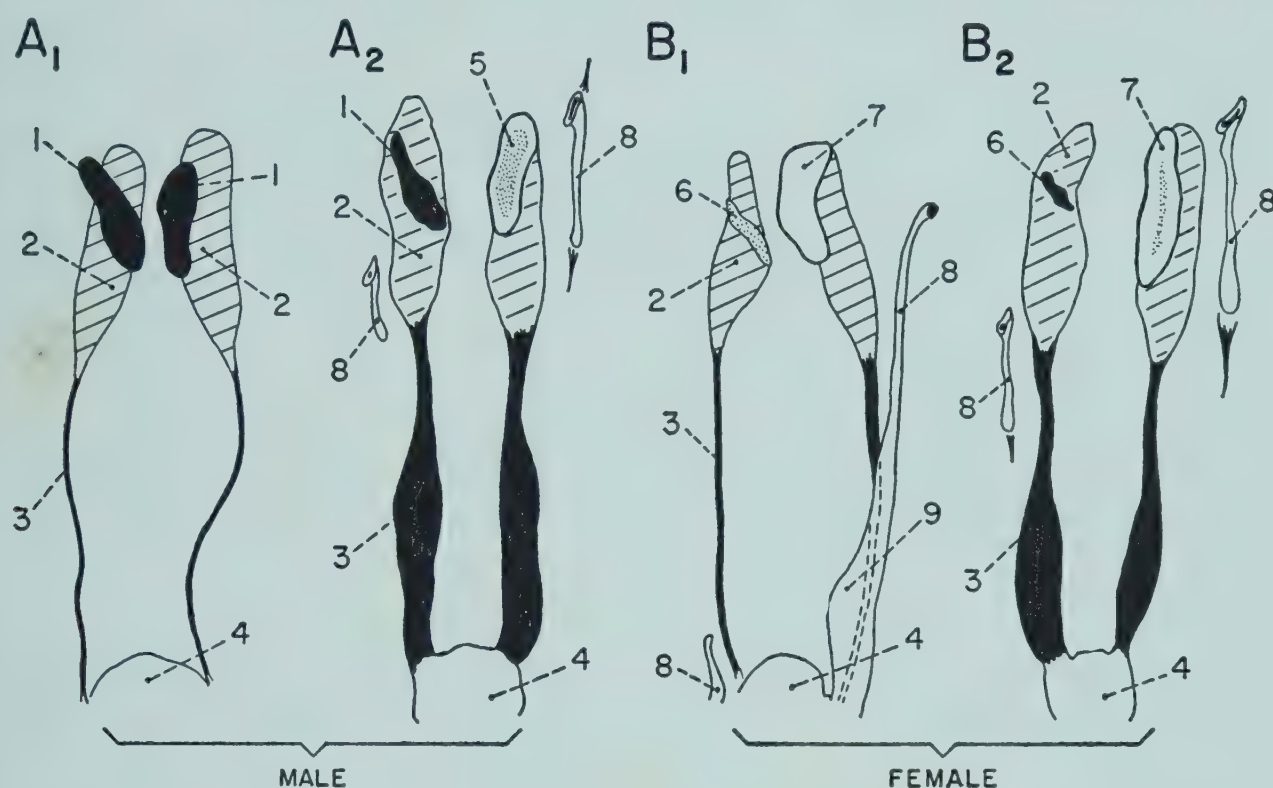


Fig. 320. Diagrammatic representation showing the effect of the male sex hormone androsterone in producing intersexes by masculinizing females and feminizing genetic males. (Redrawn with modifications after Wolff, Strudel, and Wolff, 1948.)

A<sub>1</sub>, normal male chick reproductive system; A<sub>2</sub>, male intersex after administration of androsterone showing feminizing effect—left gonad is ovotestis having oöcytes in cortex, an intermediate zone of lacunae, and testicular tubes, parts of the Müllerian ducts persist, and the Wolffian ducts hypertrophy; B<sub>1</sub>, normal female chick reproductive system; B<sub>2</sub>, female intersex after administration of androsterone showing hypertrophy of Wolffian ducts, reduction of oviducts. All  $\times 2$ .

1, testis; 2, mesonephros; 3, Wolffian duct; 4, cloaca; 5, ovotestis; 6, right ovary; 7, left ovary; 8, Müllerian duct; 9, shell gland.

### *Influence of Female Hormones*

Female hormones generally have a feminizing effect on males, both on gonads and ducts; and in females the result is a hyperfeminization. The injection of chicken eggs with estrogenic substances during the first days of the incubation period may alter the sex ratio (Table 17) in favor of females (Danchakoff, 1935a; Wolff and Ginglinger, 1935a; Breneman, 1935; Gaarenstroom, 1937; Domm, 1939). In addition, the table includes hormonal substances like testosterone and antuitrin-S which also seem to cause differentiation in the female direction. The work of Riddle and



Dunham (1942) represents a further step in this field of investigation. They administered estrogen to the ovulating female ring-necked dove (*Streptopelia decaocto*) using 0.5 or 1.0 mg. of estradiol benzoate or 2.0 or 3.0 mg. of dehydroandrosterone 36 hours before the rupture of an ovum from the ovary. The hormone was thus passed from the blood of the bird

TABLE 17  
Alteration of Sex Ratios and Production of Intersexes in Embryo Chicks  
by Administration of Hormonal Substances

Hormone	Individuals Observed (number)	Male (per cent)	Female (per cent)	Intersex (per cent)	Investigator
Alphaestradiol benzoate	356	51	49		Lewis (1946)
Alphaestradiol and theelin	51	47	53		Domm (1939)
Antuitrin-S	437	43	57		Breneman (1935)
Estrone (injected at beginning of incubation)	68	12	63	25	Gaarenstroom (1937)
Estrone (at 24 hours of incu- bation)	48	19	60	21	"
Estrone (at 48 hours of incu- bation)	45	29	60	11	"
Estrone (at 96 hours of incu- bation)	31	15	64	21	"
Estrone (at 120 hours of incu- bation)	61	8	56	36	"
Estrone (at 144 hours of incu- bation)	37	19	56	25	"
Estrone (at 168 hours of incu- bation)	82	50	45	5	"
Hexestrol	89	58	42		Lewis (1946)
Testosterone	19	43	57		Lewis (1946)



to the egg. Results were comparable to those produced by injecting eggs. Left testes had a more persistent ovarian cortex and Müllerian ducts persisted.

**Effect on Males.** The injection of estrogen, or related female hormones (Table 18), into hens' eggs during the early stages of incubation causes persistence of the left oviduct in genetic males (Fig. 321-B<sub>1</sub> and B<sub>4</sub>) and transformation of the left testis into an ovotestis as seen in Fig. 321-B<sub>2</sub>, B<sub>3</sub>, and B<sub>5</sub> (Kozelka and Gallagher, 1934; Breneman, 1935; Willier, Gallagher, and Koch, 1935c, 1937; Wolff and Ginglinger, 1935a; Danchakoff,

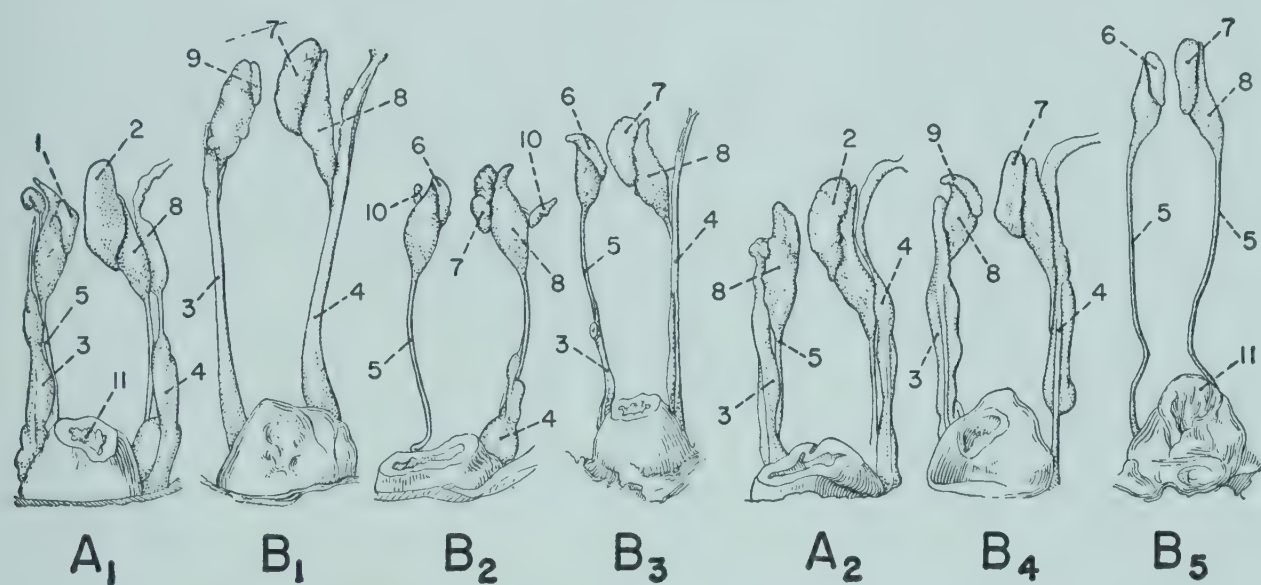


Fig. 321. The effect of two female sex hormones, theelin and theelol, showing their feminizing effect on the reproductive system of genetic male and female chick embryos. (Redrawn with modifications after Willier, Gallagher, and Koch, 1937.)

A<sub>1</sub>, theelin treated genetic female; B<sub>1</sub>, genetic male after 2.0 mg. theelin; B<sub>2</sub>, genetic male after 1.0 mg. theelin; B<sub>3</sub>, genetic male after 0.4 mg. theelin; A<sub>2</sub>, theelol treated genetic female; B<sub>4</sub>, genetic male after 1.0 mg. theelol; B<sub>5</sub>, genetic male after 0.2 mg. theelol. All  $\times 1.5$ .

1, right ovary; 2, left ovary; 3, right oviduct; 4, left oviduct; 5, Wolffian duct; 6, right testis; 7, left ovotestis; 8, mesonephros; 9, right ovotestis; 10, persisting portions of oviduct; 11, cloaca.

1936a, 1936b; Gaarenstroom, 1937, 1939; Riddle and Dunham, 1942; Masui, 1944). Comparable effects are produced by many hormonal preparations, among them hexestrol, estradiol (progynon) (Lewis, 1946), theelin, and theelol (Willier, Gallagher, and Koch, 1937), as shown in Fig. 321. An ovotestis is characterized by a cortex of ovarian type and of varying thickness, over a testicular medulla. This is usually the condition of the left gonad in an intersex, while the right gonad remains testicular.

**Effect on Females.** The hyperfeminization effect usually includes the enlargement of cortex of left and/or right ovaries and hypertrophy of the Müllerian duct (Fig. 321-A<sub>1</sub>). Either the left duct or both may be overdeveloped. In a few instances the Wolffian duct may hypertrophy as well (Fig. 321-A<sub>2</sub>).



TABLE 18

Effect of Various Female Hormonal Preparations and Other Hormones Having a Feminizing Effect on Embryonic Sex Organs and Accessory Sexual Structures

Hormonal Substance	Dosage	Day of Treatment	Day of Examination	Species	Sex	Effect				Investigator	
						Gonad		Duct			
						Medulla	Cortex	Wolffian	Müllerian		
Androsterone Antuitrin-S Dienestrol “	20-100 y 100 mg.	4 3,4 2		pheasant * f.p.	M					Danforth (1942)	
				chicken	M		female asymmetry			Breneman (1935)	
				chicken	M	f	f		f	Snedecor (1949)	
				turkey †	M	f	f		f	Jaap, Ingram, and Godfrey (1951)	
Diethylstilbestrol	0.01-0.50 mg.			chicken	M	f			f	Wolff (1939)	
“				duck ‡	M					hyper.	Lewis and Domm (1946)
“	100 mg.	2		turkey	F M	f			f	hyper. f	Jaap, Ingram, and Godfrey (1951)

Abbreviations: f	— feminizing	M	— male
F	— female	mg.	— milligrams
f.p.	— feminizes plumage	M.U.	— mouse unit
H	— hatched	R.U.	— rat unit
hyper.	— hypertrophy	unaf.	— unaffected
I.U.	— international unit	y	— gamma

Abbreviations: f — feminizing  
F — female  
f.p. — feminizes plumage  
H — hatched  
hyper. — hypertrophy  
I.U. — international unit  
M — male  
mg. — milligrams  
M.U. — mouse unit  
R.U. — rat unit  
unaf. — unaffected  
y — gamma



Estradiol “	2500 M.U. 25-500 I.U.		chicken chicken chicken	M M M F	f f	f f	Danchakoff (1935 <i>b</i> ) Wolff (1937 <i>b</i> ) Stoll (1948)
Estradiol benzoate with testosterone propionate	2 2		chicken	M	f	f	Domm (1939)
Estradiol benzoate with theelin							
Estradiol dipropionate with testosterone propionate	0.25 mg. 1.50 mg. 300-1000 y		chicken duck duck chicken	M M M M F M	f-left f-left f-left f unaf. f-left right unaf.	f f f f f-right f-right persists hyper. hyper.	Raynaud (1940) Lewis and Domm (1946) Gaarenstroom (1939) Lewis (1946)
Estradiol benzoate		9-H	duck	M			Lewis and Domm (1946)
(Progynon-B)	0.25-1.5 mg.		duck	M F			Kozelka and Gall- gher (1934) Breneman (1935)
“	100 y		chicken	M	f-left		Willier, Gallagher, and Koch (1935 <i>a</i> )
Estrone	18-25 R.U.		chicken	F	f	f	Wolff and Gin- glinger (1935 <i>c</i> )
“	56-2250 R.U.		chicken	M F	unaf. unaf.	hyper.	Gaarenstroom (1937)
“	1000- 2000 I.U.		chicken	M	f	f	
“	100 y		chicken	M	f	f	



TABLE 18 (continued)  
Effect of Various Female Hormonal Preparations and Other Hormones Having a Feminizing Effect on Embryonic Sex Organs and Accessory Sexual Structures

Abbreviations: f — feminizing F — female f.p. — feminizes plumage H — hatched hyper. — hypertrophy I.U. — international unit										M — male mg. — milligrams M.U. — mouse unit R.U. — rat unit unaf. — unaffected y — gamma
Hormonal Substance	Dosage	Day of Treatment	Day of Examination	Species	Sex	Effect				Investigator
						Gonad			Duct	
						Medulla	Cortex	Wolffian		
Estrone	50-2000 y	2	19	chicken	M	f-left	f-left		f hyper.	Willier, Gallagher, and Koch (1937) Wolff (1937b) Oordt and Rinkel (1940)
"				chicken	F	f	f		f	
"	1000 I.U.			chicken	M	f	f			
					M		unaf.			Danforth (1942)
"				chicken f.p. pheasant f.p.	M					
Gonadogen	5-30 R.U.	2-18	20	chicken	M		hyper.		f	Venzke (1941)
					F		hyper.			Lewis (1946)
Hexestrol	1.25-2.50 mg.			duck	M	unaf.	f	hyper.	f	



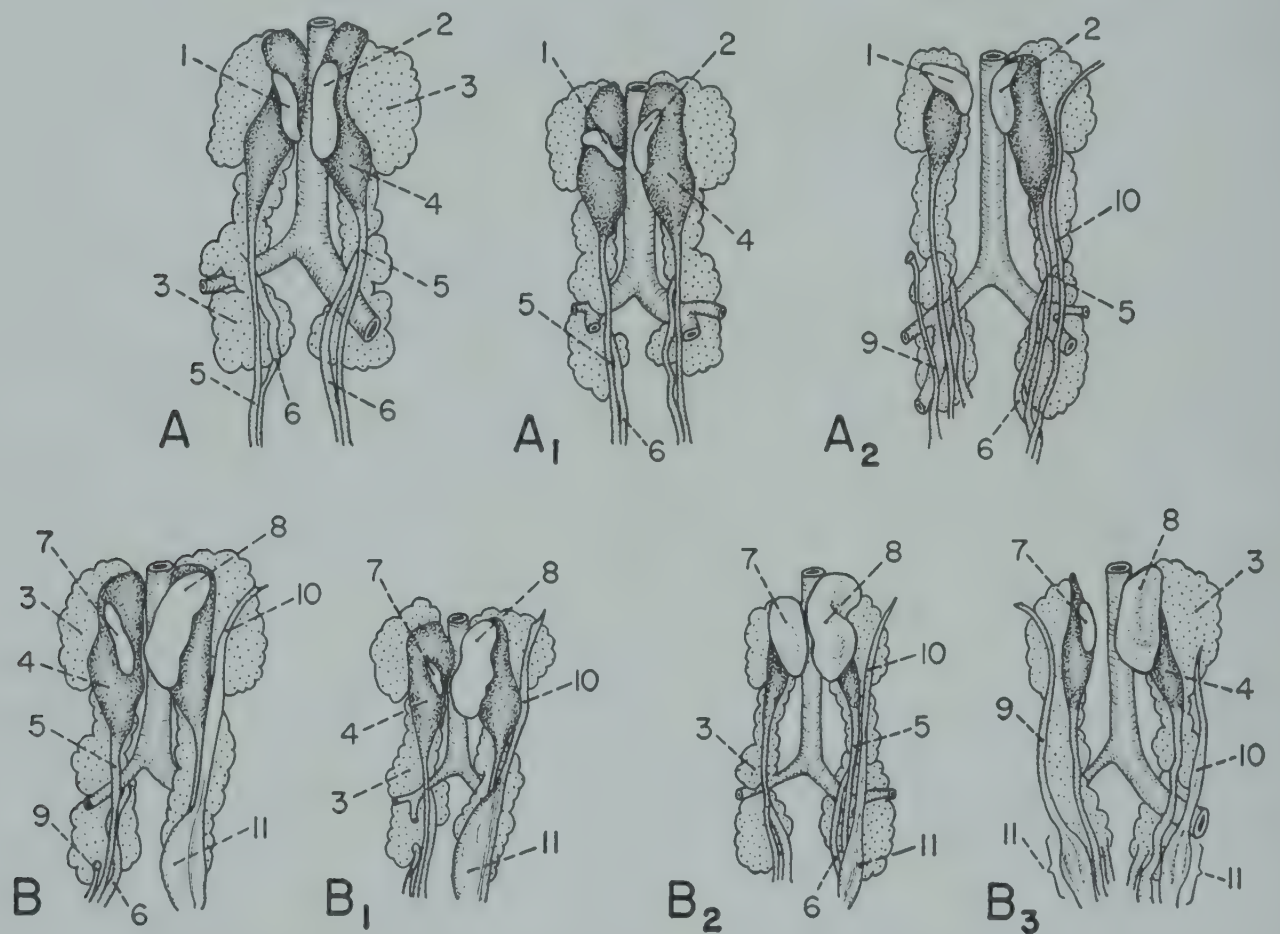
	1.25-2.50 mg.	4-8		duck	M	f			hyper. hyper.	Lewis and Domm (1946)
"	50-100 mg.	2		turkey	M	f	f		f	Jaap, Ingram, and Godfrey (1951)
N-bis-dehydro-diosynolic acid (sodium salt)	0.02-0.2 mg.	1-8		chicken	M	f	f-right		hyper.	Wolff and Wolff (1948)
Pituitary hebin	5-30 R.U.	2-9		chicken	M		hyper. left hyper.	unaf.		Domm and Dennis (1937)
Pregnant-mare serum	20 I.U.			chicken	M	hyper.	f-right	unaf.		Snedecor (1949)
Stilbestrol				chicken f.p.	M					Danforth (1942)
"	1.25 mg.	3-18	9-H	duck	M	f	f		hyper.	Lewis (1946)
"	1.25 mg.	3-18		duck	M	f	f			Lewis and Domm (1946)
"	2 mg.	3,6,9		herring gull §	M	f			f-right right and left persist cortex in right	Boss and Witschi (1947)
"				chicken	M	f				Snedecor (1949)
Testosterone propionate	30-100 y	3-6		duck	M		unaf.	unaf.		Lewis (1946)
"	1.25 mg.	3-18	9-H	turkey	M		unaf.	unaf.		Jaap, Ingram, and Godfrey (1951)
Thyroxin	1250-5000	2		chicken	M		hypoplastic	unaf.		Greenwood and Chaudhuri (1928)
"		3		pheasant f.p.	M		unaf.			Danforth (1942)

\* *Phasianus colchicus*; † *Meleagris gallopavo*; ‡ *Anas platyrhynchos*; § *Larus argentatus*.



### *Effect of Other Hormonal Substances*

**Pituitary hormones.** The removal of a gland and withdrawal of its hormone is suggestive of the effect which the hormone exerts under normal conditions. Hypophysectomy seems to indicate that the pituitary hormone (in this case, the gonadotropic factor) acts to promote normal development



**Fig. 322.** The relation of various endocrine glands to development of the embryonic chick urogenital system. (Redrawn with modifications A, A<sub>1</sub>, B, B<sub>1</sub>, after Fugo, 1940; A<sub>2</sub>, B<sub>3</sub>, after Willier and Yuh, 1928; B<sub>2</sub>, after Domm and Dennis, 1937.)

A, 18-day normal male; A<sub>1</sub>, 18-day hypophysectomized male in which testes undergo normal differentiation but are smaller than normal owing to deficiency of intertubular material, gonoducts are normal; A<sub>2</sub>, 18-day male which was host to thymus graft for 10 days, testes are normal but both Müllerian ducts persist; B, 18-day normal female; B<sub>1</sub>, 18-day hypophysectomized female in which right ovary is normal but left ovary is deficient in cortex, gonoducts are normal; B<sub>2</sub>, 18-day female which received 25 R.U. (rat unit) pituitary hebin, gonads hypertrophied, gonoducts are normal; B<sub>3</sub>, 18-day female which was host to a thyroid graft for 9 days; left ovary is normal, right ovary has cortex, and right oviduct is as well developed as normal left oviduct. All  $\times 1.5$ .

1, right testis; 2, left testis; 3, metanephros; 4, mesonephros; 5, Wolffian duct; 6, ureter; 7, right ovary; 8, left ovary; 9, right Müllerian duct; 10, left Müllerian duct; 11, shell gland.

of testes and ovaries. Extirpation of the prospective pituitary region before the end of the second incubation day has no effect on gonads or germ cells (Wolff, 1937a; Fugo, 1940). Accessory structures are not affected by the gonadotropic hormone. Hypophysectomized males are deficient in intertubular material (Fig. 322-A<sub>1</sub>), while females have a cortex deficiency in



the left ovary (Fugo, 1940) as shown in Fig. 322-B<sub>1</sub>. The injection of pituitary hebin suggests the same function—ducts are still unaffected but the gonads are overdeveloped (Domm and Dennis, 1937) as seen in Fig. 322-B<sub>2</sub>.

**Thyroid hormone.** Female embryos to which thyroid was grafted on the ninth day of incubation exhibited normal left ovaries. In some embryos, however, there was a trace of cortex on the right ovary, and the right oviduct was as fully developed as the left (Willier and Yuh, 1928) as

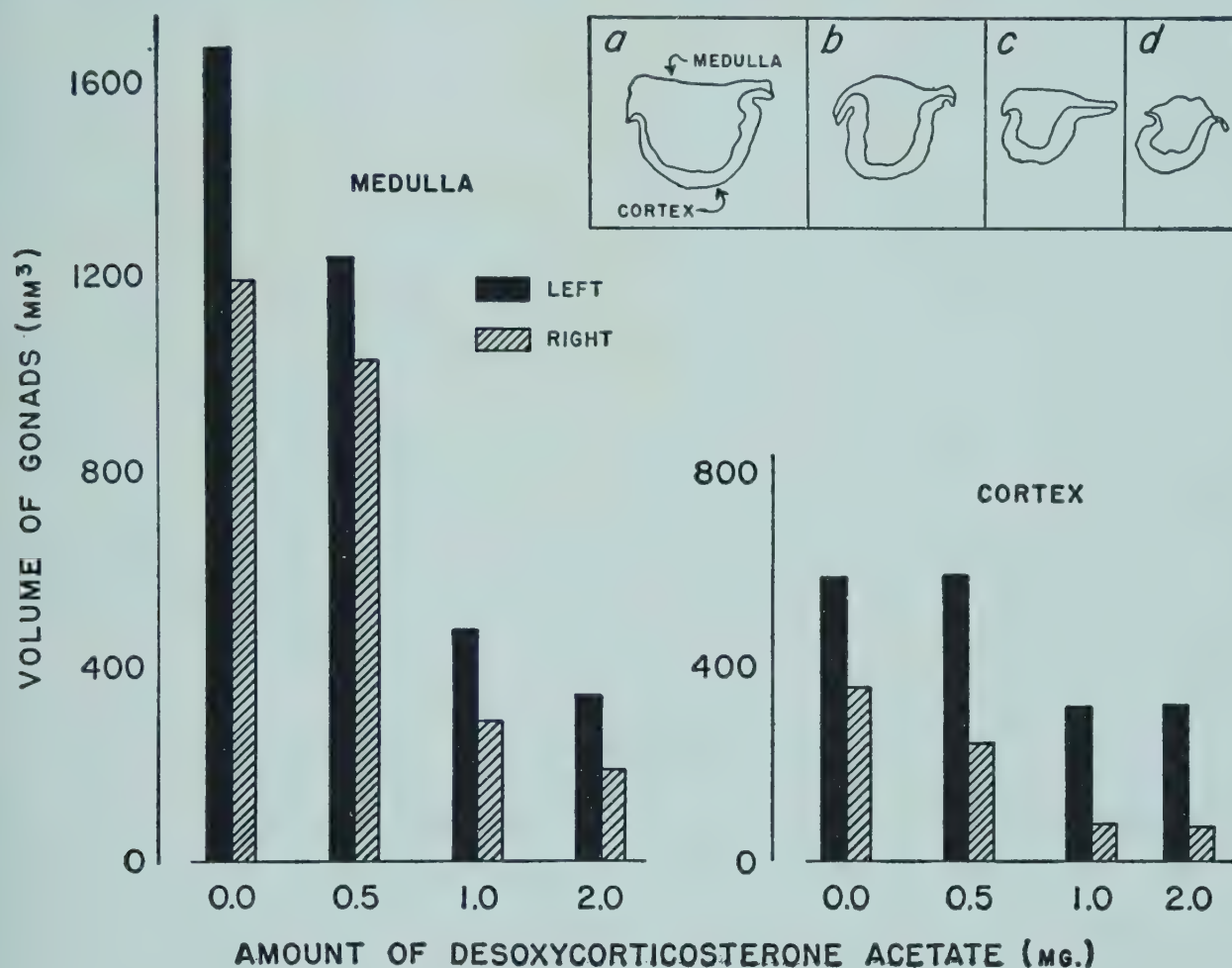


Fig. 323. The effect of desoxycorticosterone acetate on undifferentiated gonads of the chick embryo, showing volume of cortex and medulla, as affected by dosage. (Redrawn with modifications after Vannini, 1949.) All embryos treated on second day and examined on sixth day of incubation.

*Insert:* cross sections showing relative areas of medulla and cortex of four groups of observed gonads. *a*, *b*, *c*, and *d* range from control group to highest dose of hormone, respectively. All  $\times 50$ .

shown in Fig. 322-B<sub>3</sub>. A feminizing effect was also noted by Danforth (1942), who made a study of pheasants (*Phasianus colchicus*). The male plumage was transformed to a hen type by administration of thyroxine. However, injecting thyroxine into incubating 3-day chicken eggs produced no effects on the reproductive system in the experiment performed by Greenwood and Chaudhuri (1928).

**Effect of thymus.** A partial feminizing effect was achieved by Willier and Yuh (1928) by grafting a thymus for 10 days on an 8-day male embryo. Although the testes were unchanged, there was retention of the right and left Müllerian ducts, the left being the longer (Fig. 322-A<sub>2</sub>).



The development of the mesonephros, Müllerian duct, and Wolffian duct seems to be entirely unaffected by hypophysectomy (Wolff, 1937a). Later in the development of hypophysectomized embryos, the seminiferous cords are narrowed and stroma is deficient in the testes (cf. Fig. 322-A<sub>1</sub>). The left ovary is deficient in cortex and secondary cords, although the left medulla and the entire right ovary are not particularly affected (cf. Fig. 322-B<sub>1</sub>). The tendency toward the production of females following hypophysectomy was noted by Deth (1951), who obtained twenty females out of twenty-two embryos surviving this operation performed on the second incubation day.

The implantation of the hypophysis of an 8- to 20-day embryo into the body cavity of a 3- to 4-day embryo seems to have an effect on the sex of the host. Deth (1951), who performed this experiment, observed that the sex of the host usually corresponded to that of the donor.

**Adrenal hormones.** A synthetic cortico-adrenal hormone (desoxycorticosterone acetate) was administered to chicken eggs on the second incubation day, and the embryonic gonads were observed at the 6-day stage or earlier (Vannini, 1949). The gonads of treated embryos were hypoplastic (Fig. 323), mainly because of repression of medullary development, but partly because of reduced cortical growth. The right gonad was the more severely affected.

### *Effect of Other Experimental Techniques*

Castration effects, sexual reversion, and various anomalies have been produced in a variety of ways, other than through the use of hormone injections. For instance, destruction of germinal tissue by use of X-rays has proved an effective method of castration. Use of certain chemicals has produced monstrous development. There is some question as to whether or not grafts modify the sex of the host in which a sex gland of the opposite sex is transplanted.

**X-Irradiation.** The technique of X-irradiation has been used to excellent advantage in elucidating the role of the embryonic gonads and in determining the stage at which sex hormones are released into the blood circulation. The essential nature of hormonal substances in regulating sexual differentiation has been demonstrated by precocious castration of the embryo. This has been accomplished by irradiation of the whole chick embryo (Essenberg and Zikmund, 1938; Dulbecco, 1948), by intrayolk injections of radioactive phosphorus, P<sup>32</sup> (Warren and Dixon, 1949a), and by total and localized irradiation of chick and duck (*Anas platyrhynchos*) embryos (Wolff and Salzgeber, 1949; Salzgeber, 1950; Wolff and Wolff, 1951a). The result of such treatment is the production of an asexual or neutral type of accessory sex structure. Wolff and Wolff (1951a) have shown that the characteristic neutral state is identical to the male



type and that it is the result of self-differentiation of the syrinx and genital tubercle in both male and female ducks and chicks (Fig. 324). In other words, in the absence of sex hormones, the male state of the syrinx and genital tubercle is assumed, and, in addition, the oviducts persist. Thus it appears that the female hormone must be present in order to induce the

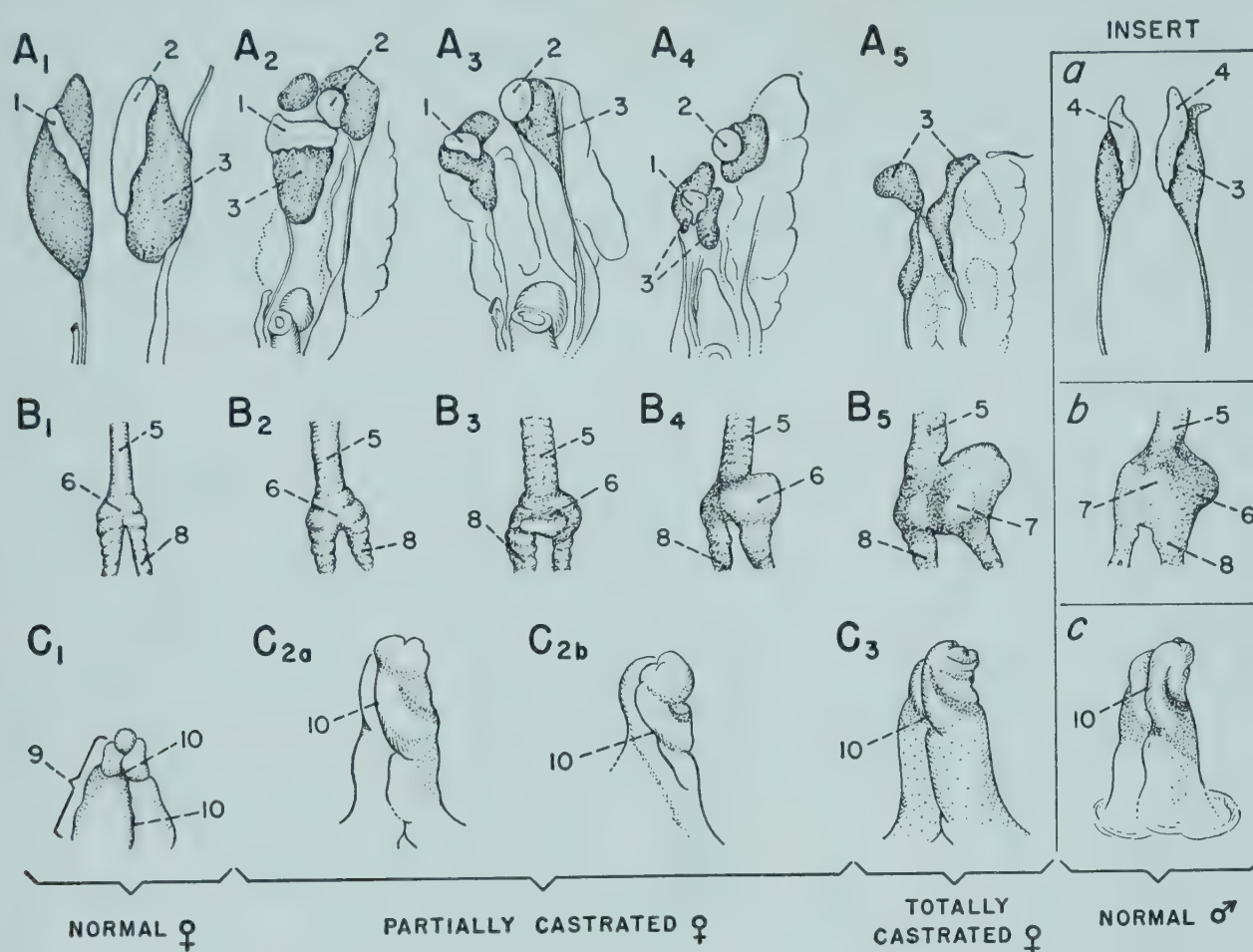


Fig. 324. The effect of partial and total castration by X-irradiation on the genital system, syrinx, and genital tubercle of female duck (*Anas platyrhynchos*) embryos. (Redrawn and modified after Wolff and Wolff, 1951a.)

$A_1$  to  $A_5$ , with decreasing amounts of female hormone the genital system receives less stimulation to differentiate into a female type and as a result it approaches a neutral (asexual), or malelike, form; all at 16 days' incubation ( $\times 2$ );  $B_1$  to  $B_5$ , with decreasing amounts of female hormone the inhibition on the neutral or male type of syrinx development is removed; thus the male form can be more fully expressed;  $B_1$ , at 14 days' incubation;  $B_2$  to  $B_5$  at 16 days' incubation ( $\times 3$ );  $C_1$  to  $C_3$ , the normal tendency of female hormone to suppress the development of the genital tubercle is lost, thus permitting the tubercle to follow its own self-differentiating tendency toward the male type which corresponds to the neutral form, all at 17 days' incubation ( $\times 6$ ).

Insert: *a*, *b*, *c*, normal male at 16, 14, and 17 days' incubation, respectively.

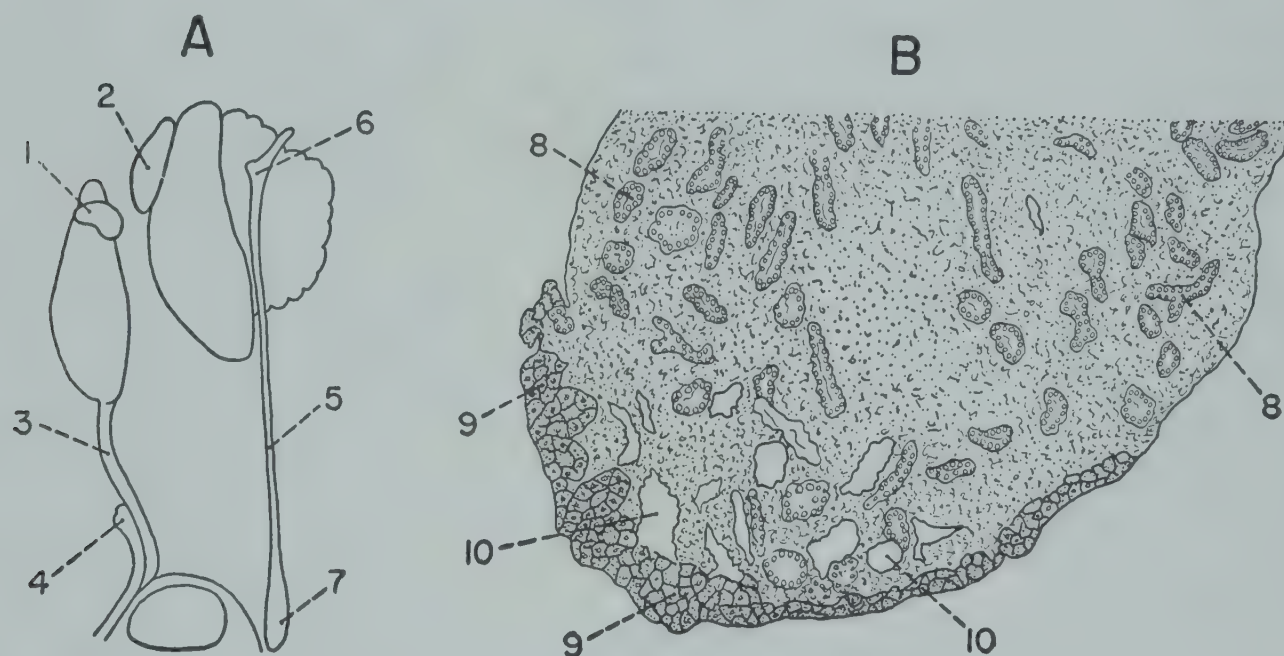
1, right ovary; 2, left ovary; 3, Wolffian body; 4, testis; 5, trachea; 6, syrinx; 7, ventral mammillary tubercle of syrinx; 8, bronchus; 9, genital tubercle; 10, seminal groove.

female form of the two secondary sex structures, and that the presence of testicular hormone is essential for the regression of the oviducts in the male. Wolff and Wolff (1951a) presented their findings as evidence that there is at first a neutral or asexual soma and that hormonal control of the differentiation of each sex is necessary to inhibit the self-differentiating tendency of certain structures of the opposite sex. Sex hormone stimulation



appears to become effective only after primary sex differentiation has occurred. The feminizing influence of ovarian grafts in the male produces intersexuality similar to that resulting from radiation (Wolff, 1949b; Wolff and Salzgeber, 1949).

Whole and partial radiation experiments on chicks have shown that germinal elements in the gonads are extremely radio-sensitive and that somatic elements also undergo injury. By irradiating chick embryos of 5- to 8-somites for 24 hours, Dulbecco (1946, 1948) found that sexual elements were completely destroyed and that cords of the second proliferation were retarded in development. All of the embryos that survived the treatment were females. In embryos treated with smaller doses, sexual



**Fig. 325.** The production of intersexes by X-irradiation. (Redrawn with modifications A, after Salzgeber, 1950; B, after Wolff, 1949b.)

A, reproductive system of genetic male chick irradiated on the sixth day of incubation and examined after 15 days of incubation ( $\times 4$ ); B, transverse section of a left ovotestis of a male intersex ( $\times 130$ ).

1, right testis; 2, left ovotestis; 3, right Wolffian duct; 4, rudimentary right Müllerian duct; 5, left Müllerian duct; 6, infundibulum; 7, uterus or shell gland; 8, testicular cord of medulla; 9, sterile ovarian cortex; 10, lacuna in intermediate zone.

elements were reduced in number and males exhibited an arrested development of cords of the first proliferation. Müllerian ducts do not undergo regression in X-ray castrated male or female embryos (Wolff, 1949a; Wolff and Wolff, 1951a). Male hormones bring about the complete regression of these ducts. In hemicastrated female embryos, the presence of female hormone from either ovary is sufficient to induce normal female development, that is, for regression of the right oviduct and growth of the left.

A certain degree of sex reversal from female to male has been induced by X-irradiation (Essenberg and Zikmund, 1938). With increasing dosages, starting at 100 r, the ovary underwent arrested mitotic activity, then showed injury to the germinal epithelium, primary follicles, and cell cords, and



finally at 300 r, exhibited destruction of follicles and the development of seminiferous-like tubules.

A series of injections of the radioactive isotope,  $P^{32}$ , given at different stages of incubation, showed that the older germ cells in the ovary are the more resistant to the treatment (*Warren and Dixon, 1949a*). Irradiation of a 4-day embryo destroyed almost all ova, while treatment at 14 days reduced the number of ova. In the male, germ cells were extremely sensitive at all stages. Whereas germ cells in at least one sex reacted quantitatively to the radiation, somatic changes were the same regardless of the dosage. In the male gonad, tubules became narrower, but columnar epithelial cells and basement membrane remained unchanged. Eventually, the epithelial cells developed vacuoles in the cytoplasm. In the ovary, the medullae were slow to show an effect, but they eventually became hypoplastic. Salzgeber's (1950) technique of irradiating only the area between the eighteenth and twentieth somite in the 2- to 6-day chick embryo converted over half of the males into typical intersexes (Fig. 325). This feminizing action of X-rays is attributed to an inhibition of male hormone secretion, thus permitting cords of the second proliferation to develop. Salzgeber suggested that the male hormone is altered to become an intermediate or female hormone.

**Chemical and Physical Agents.** Among the anomalies produced by chemical and physical agents there is the condition of pseudohyperfeminization. This state is characterized by such conditions as the disappearance of the Müllerian duct in male embryos by the fourteenth day and the development of the right Müllerian duct of females into an oviduct. These results were produced by Stoll (1945) through the use of such teratogenic substances as 1162 F (paraaminobenzene sulfamide), Albucid (paraaminobenzene sodium sulfonacetylamide), disodium methyl arsenate, sodium dimethyl arsenate, saponin and eserine salicylate, and non-teratogenic substances like saccharin, thebaine chlorhydrate, and diphtheria toxin. He also obtained this effect with sulfanilamide, arrhenal and sodium cacodylate. Raising the incubation temperature from  $38^{\circ}\text{C}$  to  $40.5^{\circ}\text{C}$  during the early part of incubation produced effects similar to the pseudohyperfeminization caused by chemicals.

**Grafting Experiments.** Evidence in support of the theory that gonad grafts do not modify host gonads is presented by Greenwood (1925), Willier (1927), Willier and Yuh (1928), and Bradley (1941). The existence of a fairly normal sex ratio is the evidence which Bradley (1941) cited to show that sex had not been modified. He did obtain intersexes, however. Greenwood (1925) repeated the work of Minoura (1921) but with different results. Hosts were embryos incubated 7 days, and the donors of the grafts varied in age from 14 incubation days to 10 weeks post-hatching. Hosts were examined in the seventeenth day of incubation. There was no evi-



dence that a grafted gonad secretes hormones which modify the genital system of the host to form an intersex, for the hosts were all of one sex or the other, irrespective of the sex of the graft, and there were no intersexes. Willier and Yuh (1928) rejected the evidence in favor of the influence of gonad grafts, since atypical sex differentiation is not unusual in embryo chicks and can be produced by low temperatures, altered humidity, and manipulation during operation.

On the other hand, the results of certain investigations have shown that the genetic direction of sexual development can be altered by grafting gonads of the opposite sex (Willier, 1952). Minoura (1921) grafted ovary or testis on the chorioallantoic membrane of the developing chick embryo and found that the developing host gonads were modified in the direction of an intersex whenever the sex of the donor differed from that of the host. Wolff (1946a, 1946b, 1946c) similarly observed reversal of primary sexual differentiation and modification of duct development apparently caused by the hormonal activity of graft implants. Grafted ovarian tissue causes formation of ovarian cortex in the left testis; and testicular tissue, although it does not affect ovarian development, suppresses the Müllerian ducts (Huijbers, 1948). It has further been established that the degree to which sex development is modified depends on the volume of tissue grafted and on the distance between graft and host gonads. Grafts close to host gonads have the greatest effect. Mintz and Wolff (1954) implanted medullary fragments of 8- to 13-day embryonic chick left ovaries into the coelomic cavity of sexually indifferent hosts of 2 to 3 days' incubation. Hosts were autopsied at 13 to 14 days' incubation. Intersexual changes in males occurred primarily in the left gonad. The right testis underwent inhibition without real feminization.

As to the growth and differentiation of the graft itself, Bradley (1941) has shown that the undifferentiated chick gonad primordia and duck (*Anas platyrhynchos*) testis primordia are self-differentiating both in homoplastic and heteroplastic combinations. Both types of grafts maintain their own rate of differentiation regardless of the sex of the host, but their rate of growth may be altered somewhat. Graft transplants in chicks were not observed to undergo any sexual modification as a result of being placed into a host of the opposite sex (Wolff, 1946a). Grafts of undifferentiated gonad rudiments were self-differentiating even when they were transplanted along with older testicular and ovarian tissue to the chorioallantoic membrane of 8- or 9-day chick embryos (Dennis, 1936). Neither the associated graft or the host sex had any effect. In rudiments originally identified as female, over half developed quite normally, and the rest showed some modifications, principally of the cortex. Some gonad rudiments gave rise to a gonad-like body of unspecific sex, a fact which Dennis attributed to factors other than the sex of the associated gonad, possibly mechanical in nature.



Ovaries and testes have been implanted in close proximity, or in contact in heterosexual combinations. Although the gonads of each sex developed normally for 8 or 9 days, no antagonistic effect of cortical and medullary components was observed (Dennis, 1936). This finding is contrary to results in amphibians, where parabiosis causes modifications due to antagonism between male and female hormones.

**Surgical Experiments.** Castration experiments (Huijbers, 1951) have provided evidence of (1) the role of male hormone in reducing the Müllerian duct, (2) the effect of female hormones in suppressing the testicular elements of the right ovary and in promoting development of the left oviduct, and (3) the action of the right gonad in suppressing the right Müllerian duct. After left castration of male chick embryos, Müllerian ducts are only incompletely reduced at hatching time; after total castration of males, both Müllerian ducts are well developed and possess shell glands; after left castration of females, the right ovary is hypertrophied by increase of medullary cords (no actual formation of testicular tubules) and the right Müllerian duct is normally reduced; after right castration of females, both Müllerian ducts are equally well developed.

### Sex Ratio

Sex ratios among domestic fowl and among chickens in particular have been extensively recorded and attest to a variable state of balance, as shown in Table 19. Crew (1937) concluded that the heterogametic sex in birds—the female sex—has a higher prenatal death rate, and that a factor is immediately found in the heterogametic mechanism. The data compiled from the literature by Lambert and Curtis (1929), mostly on adult chickens, indicate a slight excess of females, however.

In hybrid crosses of pigeons (*Columba livia*) there is a marked differential death rate among embryos. Out of 575 fertile eggs, for example, there was a high mortality in the first few days of incubation, after which 222 males and three females continued to develop (Cole, Painter, and Zeimet, 1928b). This represents a special case, however, involving incompatibility of parental chromosomes in hybrid zygotes.

Mayr (1939) recognized the occurrence of unusual sex ratios in wild birds; but reports of sex ratios in adult wild birds are often misleading. The work of McIlhenny (1940) is one of the few studies in which sex ratio was determined by observation of nestlings, and only in those nests in which the average complement of eggs hatched. An extremely unbalanced sex ratio was found in both the boat-tailed grackle (*Cassidix mexicanus*) and the blackbird (*Agelaius phoeniceus*). No satisfactory explanation of unbalanced sex ratios has been forthcoming. Mayr (1939) concluded only that there is every reason to believe that sex-linked lethals are not operative, and that we do not know enough about physiological factors influencing sex ratios. Spontaneous sex reversal is rare in nature. Diversity in adult ratios can be attributed to environmental factors.



TABLE 19  
Observations on Sex Ratio of Various Species of Avian Embryos  
Prior to, or at Hatching

Species	Number Observed (alive or dead)	Sex Ratio (% males)	Investigator
Chicken ( <i>Gallus gallus</i> )	326 (alive)	56.4	Asmundson (1941)
“ “	1,769 (dead)	47.9	Asmundson (1941)
“ “	96,008 (alive)	49.2	Byerly and Jull (1935)
“ “	6,864 (dead)	48.6	Byerly and Jull (1935)
“ “	420 (alive)	45.2	Crew and Huxley (1923)
“ “	218 (alive)	46.3	Domm and Dennis (1937)
“ “	453 (cross)	47.9	Domm and Dennis (1937)
“ “	870 (alive)	49.7	Hays (1945)
“ “	8,355 (alive)	49.8	Hazel and Lamoreux (1946)
“ “	1,247 (dead)	47.5	Lambert and Curtis (1929)
“ “	8,113 (alive)	44.5	Landauer (1943)
“ “	909 (defective)	49.3	Landauer (1943)
“ “	67,993 (alive)	48.8	Landauer and Landauer (1931)
“ “	1,851 (alive)	48.3	Pearl (1917a)
“ “	805 (alive)	47.8	Thomsen (1911)
Total	196,401		
Average		48.5	
Turkey ( <i>Meleagris gallopavo</i> )	515 (alive)	55.3	Asmundson (1941)
“ “	6,867	51.5	Asmundson (1941)
Pheasant ( <i>species crosses</i> )	766 (alive)	67.1	Thomas and Huxley (1927)
Grouse ( <i>Bonasa umbellus</i> )	464 (alive)	51.5	Bump, Darrow, Ed- minster, and Crissey (1947)
Pigeon ( <i>Columba livia</i> )	81 (dead)	54.3	Cole and Kirk- patrick (1915)
Homing pigeon ( <i>Columba livia</i> )	136 (alive)	53.7	Cuénot (1900)
Boat-tailed grackle ( <i>Cassidix mexicanus</i> )	420 (alive)	30.3	McIlhenny (1940)
Blackbird ( <i>Agelaius phoeniceus</i> )	412 (alive)	76.6	McIlhenny (1940)

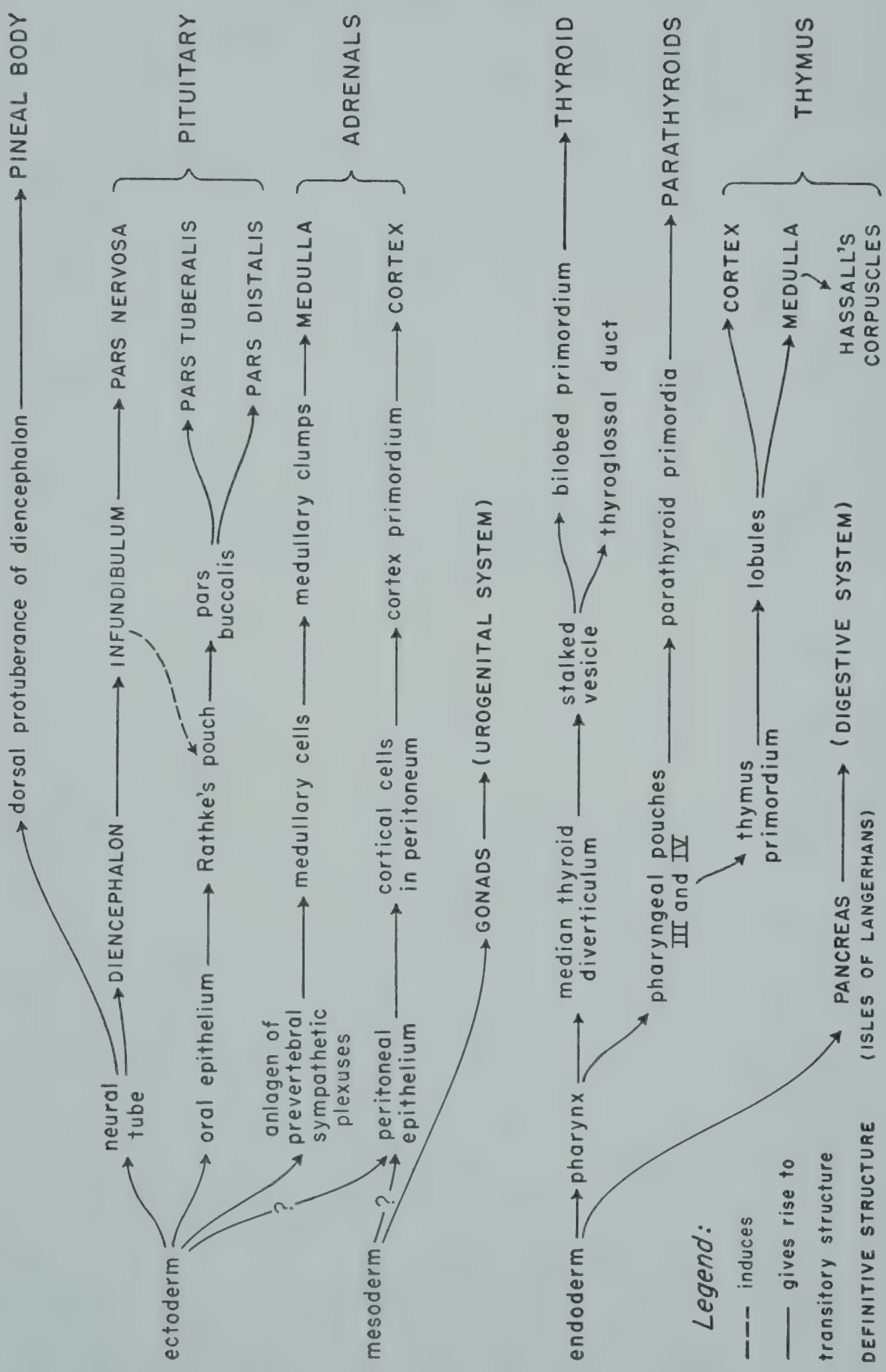


## CHAPTER ELEVEN

# *The Endocrine Glands*

*The endocrines are active centers  
Of regulation and control;  
As growth reducers and augmenters,  
They change the structure as a whole.*





DEVELOPMENT OF THE ENDOCRINE SYSTEM IN THE AVIAN EMBRYO



## THE ENDOCRINE GLANDS

The endocrine system of an organism is responsible for the coordination of general metabolic processes and the regulation of the development and function of many organs of the body. The intricate and delicate coordinating mechanism is dependent on the action of hormones which are released by the glands of internal secretion. The hormones are specific organic substances which may be released into circulating fluids and exert an effect on tissues anywhere in the body, or which may simply diffuse from the cells of origin into surrounding tissues. Typically, the hormones are effective in small quantities without actually contributing significant amounts of substance or energy. They probably act by modifying the course of basic enzymatic reactions, but there is no proof that they actually participate in enzyme systems.

The most active ductless glands during avian embryogeny are the thyroid, the adrenal, and the hypophysis, but there is a surprising paucity of knowledge concerning endocrine relationships in the bird embryo. The role of the hypophysis is highly important in general growth and differentiation and exerts complex effects, especially on bone, muscular, and nervous tissues. Equally significant, however, is its regulation of other endocrine organs. The pituitary produces certain principles which act specifically on the thyroid, adrenals, pancreas, and gonads. Probably not all of these are active during embryonic states, but little work has been done to clarify this problem. If we draw from the data on mammalian and amphibian embryos, we can be reasonably certain that the thyroid is important in the regulation of basal metabolism and exerts its influence by producing thyroxin.

The suprarenal glands are active in hormone production early in embryonic life, but it is not clear when they begin their manifold vital functions, including fluid balance, electrolyte balance, kidney regulation, and others. The hormones of the testis and ovary do not become significant until after hatching, except as they influence development of parts of the reproductive tract. Very little is known about the role of the parathyroid, the thymus, the pineal gland, and the pancreas in the embryos of birds.

It is quite certain that endocrine glands take no part in the early determination of the embryonic organs, for the differentiation of these glands starts after organ determination is completed. However, many embryonal



histogenetic processes occur somewhat later and could very well be influenced by endocrine factors. This is suggested in the work of Fugo (1940), which shows that plumage is affected by the thyroid. The morphogenetic role of the sex glands is clear from the marked effect of their secretions on the development of secondary sex characteristics.

In postnatal life, the regulatory function of the endocrine glands is of much greater significance than the morphogenetic function. Whenever a deviation from the optimum occurs, the endocrine system can be mobilized as one of the most important regulators of vital functions in the organism. But, as Studitskii (1946) pointed out, the relatively constant internal activity during embryonic life greatly decreases the need for the endocrine regulation of basal metabolism. Since there is slight need for endocrine control of possible disorders occurring during the initiation of metabolic balance, the activity of the glands is very limited.

Evidence of the role of endocrine factors in embryonal development is based mainly on the morphological signs of functional activity on the hormonal contents of the gland, and on observations of abnormal development resulting from endocrine disturbances. Thomas, E. (1933) concluded, on the basis of such evidence, that the endocrine glands of higher vertebrates do not affect development during early embryonal stages. Their influence on embryonal development starts during the later stages, and then they act only to accelerate or delay the process of development. Most endocrine glands, according to Thomas, begin their activity in the postnatal period, although they are able to function at the end of incubation.

## THE THYROID GLAND

The thyroid gland is one of the essential endocrine organs and has been widely studied among the vertebrates. It has been repeatedly investigated in embryonic as well as adult amphibians, birds, and mammals. The avian thyroid is a bilobed structure lying ventral to the pharynx and situated near the base of the neck. It is derived from an evagination of the floor of the pharynx, and it attains its follicular structure and begins its secretory function by the middle of the incubation period.

The gland is composed of follicles, each consisting of a colloid-containing lumen surrounded by a single layer of cuboidal epithelial cells. An extensive vascular supply is provided to the acinous mass of follicles.

The principal role of the thyroid is that of maintaining a generally normal metabolic rate. The action of the gland is best demonstrated by experiments in which the gland is extirpated and symptoms of hypothyroidism are produced, or in which additional thyroid material is grafted and an excess of thyroid hormone is released into the system of the host.

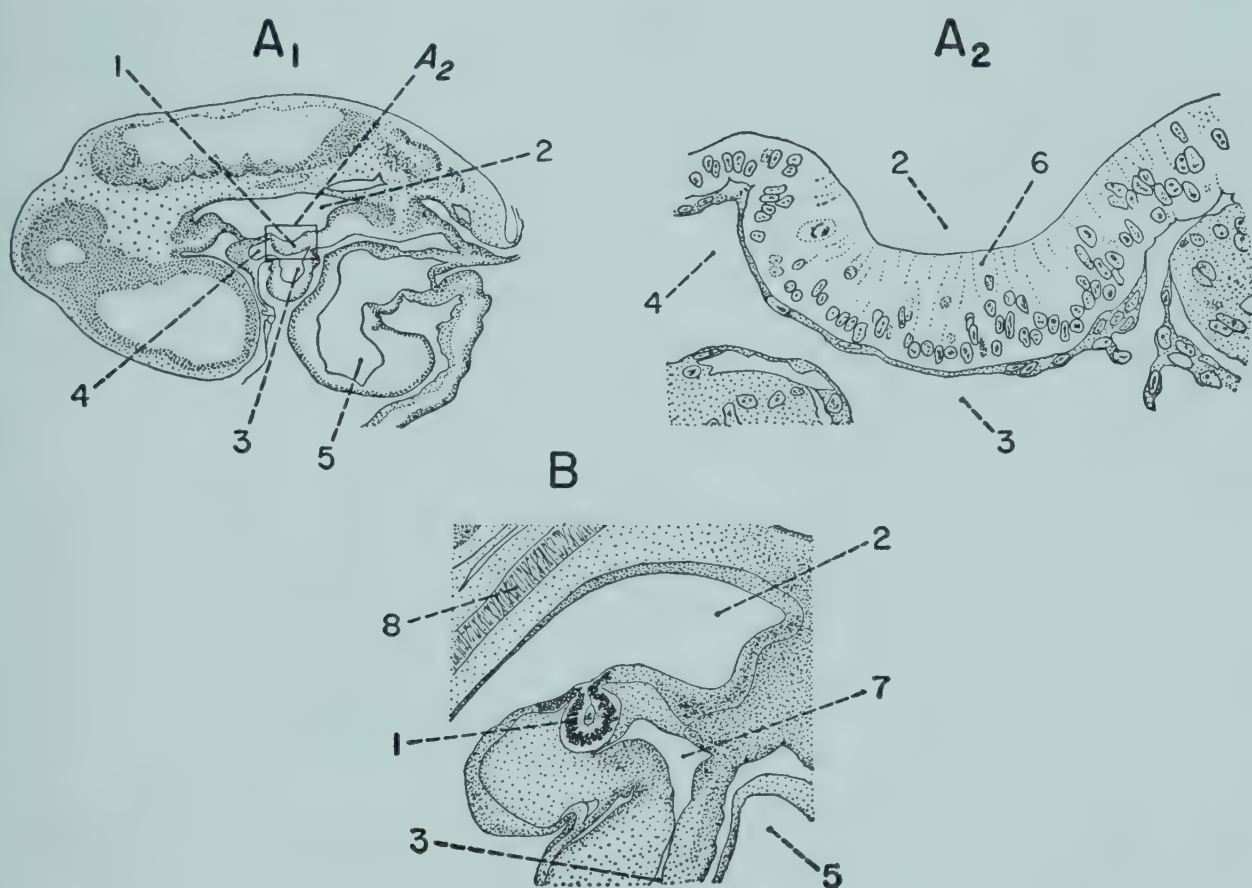


### Developmental Morphology

The thyroid is one of the first of the endocrine glands to develop in the embryo. On the chick's second day of incubation, it appears as a median outpocketing of the pharynx but soon becomes paired. By the fifth day, it is already a two-lobed structure moving to its definitive position, and by the time of hatching, it is functioning and nearly fully formed.

#### Early Embryogeny

**Localization of thyroid-forming tissue.** The thyroid-forming potency of the early blastoderm becomes evident when pieces of blastoderm are



**Fig. 326.** Early development of the thyroid gland in the chick embryo. (Redrawn with modifications after Yoshikawa, 1930.)

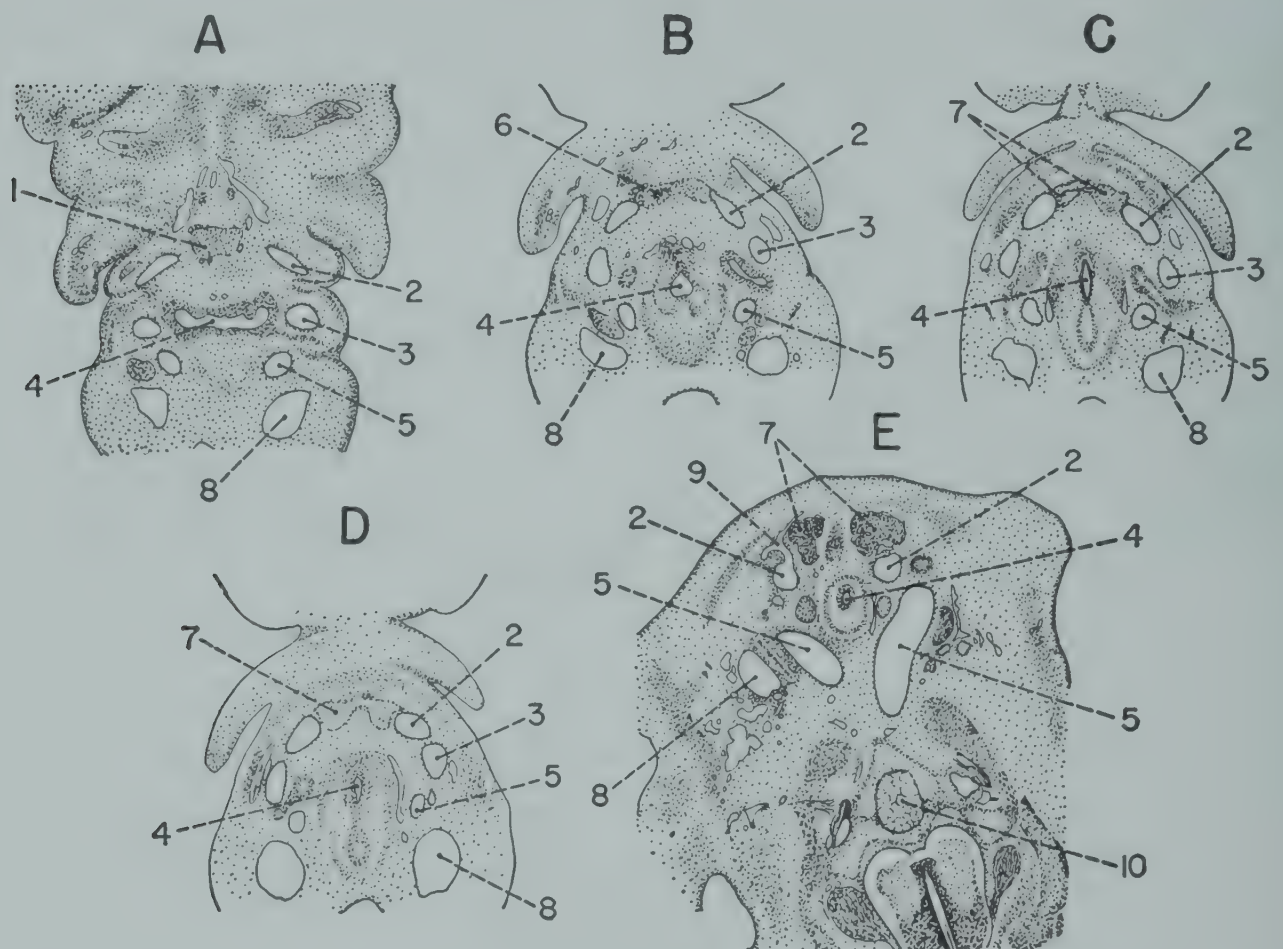
A, parasagittal section of 2.5-day embryo showing position of thyroid anlage ( $\times 20$ ); A<sub>2</sub>, detailed diagram showing columnar cell structure of thyroid within area outlined in A<sub>1</sub> ( $\times 200$ ); B, sagittal section of 3-day embryo ( $\times 50$ ).

1, thyroid anlage; 2, pharynx (esophagus); 3, truncus arteriosus; 4, first aortic arch; 5, heart; 6, columnar epithelium cell; 7, ventral aorta; 8, notochord.

grown as chorioallantoic grafts (Rudnick, 1932). At the primitive streak and early head-process stages, thyroid-forming potency is present in right, median, and left pieces of the anterior end of the blastoderm. After the formation of the head fold, only lateral pieces of blastoderm are capable of developing into thyroid. This change indicates a bilateral localization or a lateral division of the chick's thyroid anlage after 72 hours of incubation.



**Early morphogenesis.** There have been many accounts of the origin and development of the thyroid gland, but there has always been complete agreement on its origin as an evagination from the floor of the pharynx (Remak, 1855; Müller, 1871; Seessel, 1877; Kölliker, 1879; Mall, 1887; Bradway, 1929; Yoshikawa, 1930; Hopkins, 1935; Venzke, 1949). The primordium is located in the region of the bulbus aortae at the level where the latter divides into the two ventral aortae (Fig. 326-A<sub>1</sub>). The time of appearance of the evagination has been variously reported by different



**Fig. 327.** Development of the bilobed structure of the thyroid gland shown in frontal sections of the chick embryo. (Redrawn with modifications after Yoshikawa, 1930.)

A, 4-day 6.5-hour embryo in which thyroid is a single median body located between the third aortic arches; B, 4-day 7-hour embryo; C, 4-day 8-hour embryo; D, 4-day 10-hour embryo; E, 5.5-day embryo. All  $\times 50$ .

1, median thyroid gland; 2, third aortic arch; 3, fourth aortic arch; 4, esophagus; 5, sixth aortic arch; 6, lumen in elongating thyroid; 7, thyroid gland; 8, jugular vein; 9, subclavian artery; 10, notochord.

observers. Venzke (1949) noted a spherical protrusion of pharyngeal epithelium in the 30- to 36-hour chick. Seessel (1877) reported the first indication of the thyroid to be present at the end of the second day of incubation. The primordium has been seen in the chick incubated 42 hours (Yoshikawa, 1930), 54 hours (Bradway, 1929), and 3 days (Remak, 1855; Hopkins, 1935). At first the gland consists merely of a single layer of high columnar cells (Fig. 326-A<sub>2</sub>). There are, however, two zones, an inner cytoplasmic and an outer nucleate zone (Yoshikawa, 1930). The gland has



been described at this stage as a saucer-shaped depression (Venzke, 1949) projecting from the median sagittal level of the pharyngeal wall and measuring 0.12 to 0.17 mm. (Müller, 1871; Kölliker, 1879). At 84 hours the chick's thyroid is a saclike vesicle (Fig. 326-B).

Although the thyroid starts as a median outpocketing of the pharynx, it soon loses connection with the digestive tract and divides to become a bilobed structure. On the fourth day the epithelial protrusion constricts from the pharynx wall, to which it remains attached, however, as a stalked vesicle (Venzke, 1942). While it is still attached to the pharynx, the connection (thyroglossal duct) is a solid strand, two cells in diameter (Hopkins, 1935). According to Yoshikawa (1930), the thyroid becomes bilobed during the fourth day. From its nearly rectangular shape (Fig. 327-A), it constricts in the middle and elongates, approaching the third aortic arches, between which it lies (Fig. 327-B). An hour later, the constriction becomes deeper (Fig. 327-C). The two lobes are almost separate and at the same time they move anteriorly (Fig. 327-D). On the following day, the lobes of the paired thyroid gland are anterior to the third aortic arches and the gland is lobulated because of extensive vascularization. The blood supply is received through the subclavian arteries coming from the third aortic arches (Fig. 327-E). In the meantime, the thyroglossal duct has become a vestigial isthmus of cells. Remak (1855), Bradway (1929), Hopkins (1935), and Venzke (1949) observed that the transformation of the bilobed state takes place on the fifth day of chick incubation. At this time, the thyroid increases steadily in size. The measurements given in Table 20 indicate its growth.

TABLE 20

The Growth of the Developing Chick's, *Gallus gallus*, Thyroid Gland \*

Incubation Stage	Length of Gland	Depth of Gland	Distance to Gut	Distance to Lower Jaw	Thickness of Epithelium at Point of Evagination
(day)	(microns)	(microns)	(microns)	(microns)	(microns)
Early third	167	83	417		27
Late third	170	120	800	400	42
Early fourth	183	125		428	45
Late fourth	208	176		666	
Fifth	209	250		750	

\* After Seessel (1877).

From the sixth to the eleventh day mesenchyme invades the gland. On about the sixth day the parathyroid primordium is in very close approxi-



mation to that of the developing thyroid (Fig. 328-A<sub>1</sub>). At this time the gland is syncytial and nonfollicular spaces are scattered throughout (Fig. 328-A<sub>2</sub>). The invading mesenchyme crowds the glandular epithelium into plates or cords, and the circulatory sinusoids are still present (Fig. 328-B<sub>1</sub>) (Bradway, 1929; Yoshikawa, 1930; Hopkins, 1935; Venzke, 1949). Carpenter (1942) described the 8-day chick thyroid as ranging in length from 0.34 to 0.40 mm. and consisting of convoluted cords of cells, blood spaces,

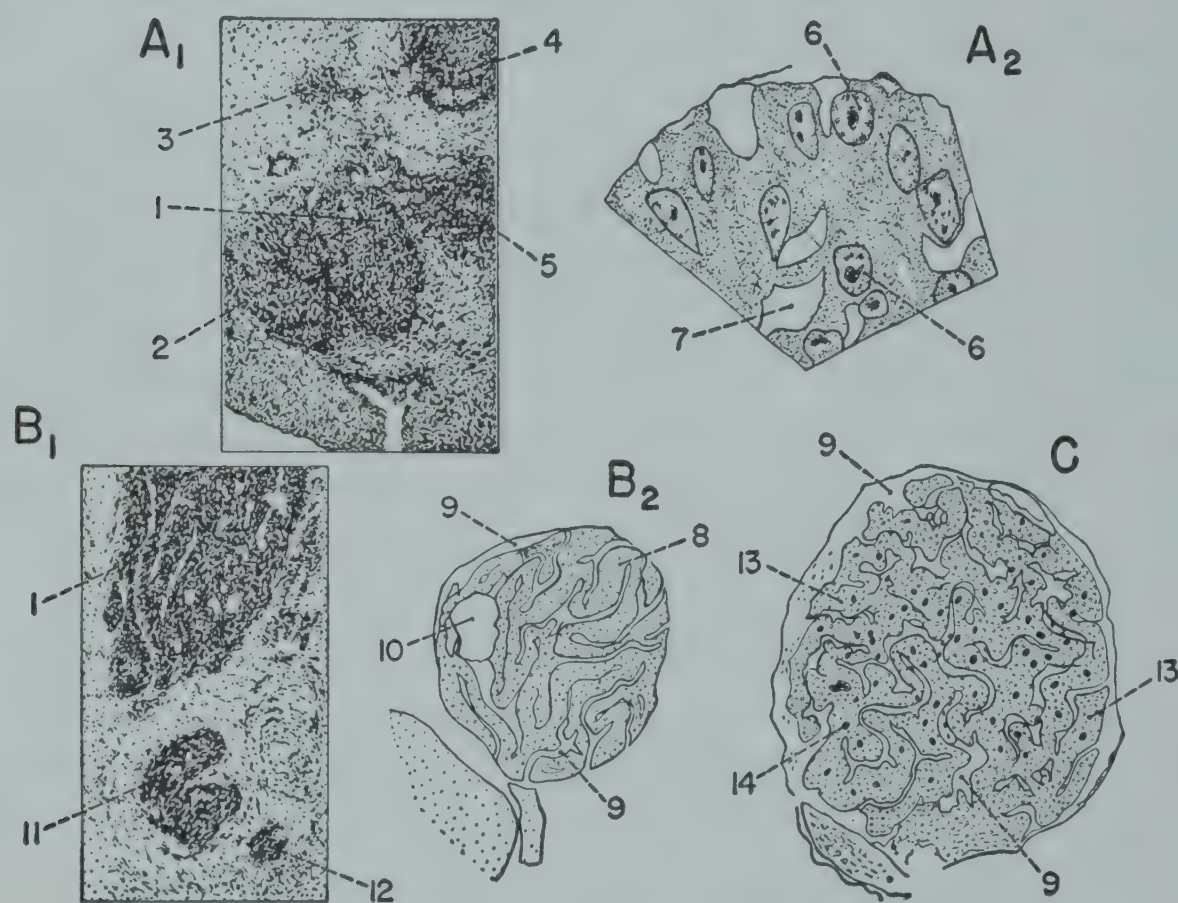


Fig. 328. Structural development of the thyroid gland between the sixth and thirteenth days of incubation in the chick embryo. (Redrawn with modifications A<sub>1</sub>, B<sub>1</sub>, after Venzke, 1949; A<sub>2</sub>, after Bradway, 1929; B<sub>2</sub>, C, after Hopkins, 1935.)

A<sub>1</sub>, sagittal section of embryo at 6.5 days, showing the relative positions of the thyroid and parathyroid primordia ( $\times 60$ ); A<sub>2</sub>, detailed drawing of thyroid tissue ( $\times 1800$ ); B<sub>1</sub>, sagittal section of 8-day 20-hour embryo, with thyroid tissue in epithelial cords ( $\times 60$ ); B<sub>2</sub>, details of gland at 8 days ( $\times 350$ ); C, histological structure of gland at 13 days ( $\times 350$ ).

1, thyroid lobe; 2, parathyroid primordium; 3, thymus primordium; 4, esophagus; 5, trachea; 6, thyroid tissue nucleus; 7, nonfollicular space; 8, epithelial cord; 9, circulatory sinus; 10, vein; 11, anterior lobe of parathyroid; 12, posterior lobe of parathyroid; 13, plate of epithelial cells; 14, colloid droplet.

and traces of connective tissue. According to Hopkins (1935) the 13-day gland still consists of epithelial plates but colloid droplets begin to appear in them. A vascular sinus surrounds the entire gland (Fig. 328-C). Kölliker (1879) described the gland on the ninth day of incubation as being a network of solid cylindrical cords 15 to 25 microns thick. By the twelfth day, each cord acquires a lumen which soon dilates. The spherical follicles, ranging in diameter from 12 to 20 microns, now appear next to the tubules (Kölliker, 1879). Bradway's (1929) observations on follicle formation



indicated that the tissue of the prefollicle is a coiled, fenestrated membrane, two cells thick. Before follicle formation, the fenestrated membrane breaks up and the cells rearrange into spherules of long conical cells.

### *Period of Growth and Function*

The thyroid gland begins functioning midway through the incubation period. By this time the follicles are fully differentiated, but further specialization occurs in that two types of colloid are elaborated.

**Beginning of function.** The appearance of colloid in the follicles of the thyroid indicates that glandular function is beginning. Colloid droplets were observed in the thyroid of the 10-day chick embryo (Sun, 1932; Hopkins, 1935; Martindale, 1941; Carpenter, 1942). Chromophilic colloid, which has an affinity for stains, is the first to appear. Venzke (1942) noted the first intracellular chromophobic (nonstaining) colloid at 240 hours, the first primary (definitive) follicle at 260 hours, and the appearance of chromophilic colloid in the follicles at 295 hours in the chick. Because of the increased secretion of droplets of chromophobic colloid in the late embryonic stages, it is thought that the function of this colloid is to dilute the more dense chromophilic colloid (Venzke, 1949). Bradway (1929) observed the first appearance of primary follicles and prefollicular globules at 11 days. Prefollicular globules are colloid droplets which appear even before follicles are well defined. The latter stained lightly. Primary follicles do not have distinct borders but are organized by the clustering of nuclei around prefollicular globules of chromophile colloid (Fig. 329-A). Yoshikawa (1930) noted colloid at 11 days. On the basis of the initial secretion of injected radioiodine ( $I^{131}$ ) by the thyroid follicles of the embryo chick, Hansborough and Khan (1950) concluded that secretory function begins on the eleventh day. According to Stoll and Blanquet (1953) the deposition of iodine begins at 10 days' incubation.

The secreting follicles are lined by cuboidal cells with peripheral nuclei. Bradway (1929) stated that the primary follicular cavities (which are not hollow, but are colloid-filled), are formed as a result of the secretion and coalescence of globules of chromophobe colloid (Fig. 329-B). These globules have their origin in the follicular cells and are produced on the side of the cell next to the lumen, near the bean-shaped nuclei (Venzke, 1949) (Fig. 329-C). Follicles are typically composed of six to seven cells.

**Morphogenesis During the Latter Half of Incubation.** Beginning on the thirteenth day, all stages of follicle formation can be seen in the thyroid (Carpenter, 1942; Venzke, 1949) as shown in Fig. 329, including stages typical of the adult gland. Chromophilic colloid is present in large follicles, and it may contain droplets of chromophobic colloid. Some cells may produce one type of colloid while other cells secrete the other type.

Venzke (1949) measured the diameters of follicles from basement



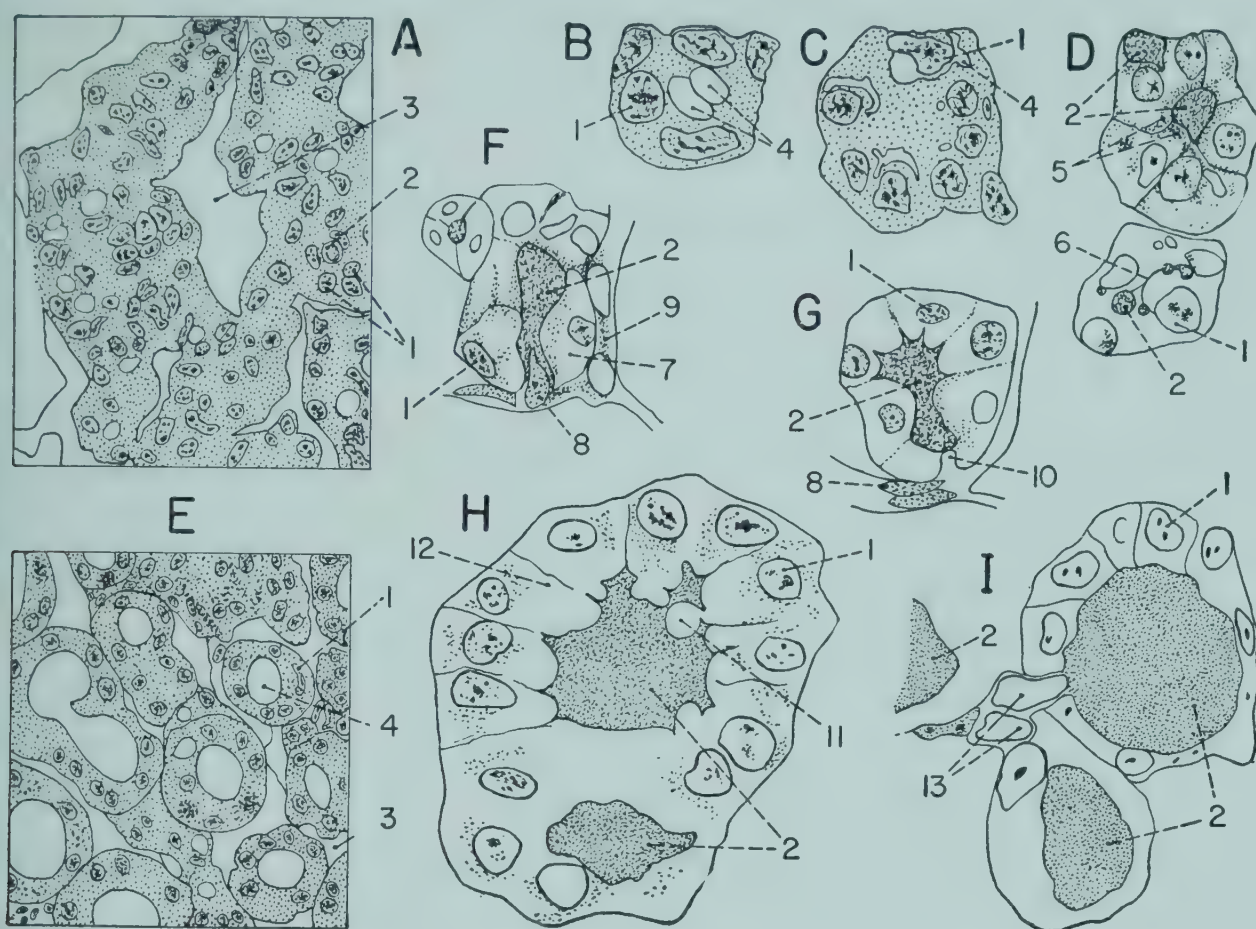
membrane to basement membrane to demonstrate the increase in size from the twelfth day of incubation to hatching. Twenty embryos were sampled in each age group shown in the accompanying table.

Age of Chick Embryo (days)	Diameter of Follicle (microns)
12	12.8
13	13.5
14	14.8
15	17.6
16	18.7
17	20.5
18	22.8
19	22.0
20	21.0

On the thirteenth day, the interior of the gland is a large sinus which is connected to the circulatory spaces which ramify between the convoluted epithelial plates (*Hopkins, 1935*). At 14 days, the follicles are lined with high columnar cells in which secretion granules collect (Fig. 329-D). After the fifteenth day, the follicles grow in size and number. Size increase results from the fusion of follicles as well as from the proliferation of epithelial cells. According to *Bradway (1929)*, the large follicles are ovoid, saclike vesicles occurring in groups of two or three, closely packed. In the 16-day chick embryo (Fig. 329-E), traces of cordlike structures can still be seen (*Carpenter, 1942*). At this time, most follicles are in contact with the vascular spaces, and tubelike openings occur in the follicular wall (Fig. 329-F). *Venzke (1949)* observed that the largest follicle in the 17-day embryo's thyroid measures 30 microns in diameter, whereas the average follicular height is 8 microns. A day later, many single, tubelike openings connect the follicle lumen to the surrounding circulatory spaces (Fig. 329-G). The basement membrane remains intact, and colloid does not seem to escape through the openings (*Venzke, 1949*). High columnar epithelium is associated with increased activity, and at the same time, rounded apical ends of epithelial cells indicate lack of tension within the follicles (Fig. 329-H). Near the time of hatching, the interfollicular cells are larger than those in follicles. The nuclei of the former are basophilic and the cytoplasm is nongranular. In general, the tissue of the thyroid gland around hatching time is compact, and much of the vascular space is crowded out of existence. The endothelial lining of the vascular spaces remains between the follicles and forms a basement membrane for the follicular cells. Near the periphery of the gland, large vascular spaces persist. Erythrocytes are present in this region. Meanwhile the gland is enclosed by a thickening connective tissue capsule from which fine trabeculae extend into the parenchyma.



*The thyroid at the time of hatching.* Thyroid weight and size are quite variable at the time of hatching, as shown by the figures compiled by Aberle and Landauer (1935). In 900 newly hatched chicks, the average thyroid weight was 12.8 mg. in male chicks and 14.1 mg. in female chicks.



**Fig. 329.** Morphogenesis of thyroid follicles in the chick embryo, showing characteristic conditions which occur during the developmental period. (Redrawn with modifications A, B, C, and E, after Bradway, 1929; D, F, G, H, and I, after Hopkins, 1935.)

A, section through end of thyroid gland of a 12-day embryo ( $\times 150$ ); B, follicle from an 11-day embryo showing two globules of colloid, about to coalesce, lying between two bean-shaped nuclei ( $\times 900$ ); C, follicle from same age embryo showing globule of colloid near an irregular nucleus ( $\times 900$ ); D, two follicles from a 14-day embryo ( $\times 750$ ); E, section through part of gland of a 16-day embryo ( $\times 400$ ); F, follicle from a 17-day embryo, with endothelial cell partially blocking the drainage tube for the colloid ( $\times 750$ ); G, the same, with branch from a circulatory space almost in contact with the colloid ( $\times 750$ ); H, the same, showing the secretion of a chromophobe colloid droplet into the follicle lumen ( $\times 750$ ); I, follicle from a 19-day embryo ( $\times 750$ ).

1, follicle cell nucleus; 2, chromophile colloid; 3, nonfollicular space; 4, chromophobe colloid; 5, secretion granules; 6, line of colloid seepage; 7, drainage tube; 8, endothelial cell; 9, colloid in circulatory space; 10, branch of circulatory space; 11, chromophobe droplet; 12, high columnar epithelium cell; 13, erythrocyte.

The definitive position of the thyroid gland in birds was described by Forsyth (1908), who examined the thyroids of thirty-four different species of birds, mostly adults. The general location is nearly the same as in the embryo at the middle of the incubation period, that is, along the internal jugular vein at the junction of the subclavian and carotid arteries



(Sun, 1932). In birds, the thyroid is never in close contact with the trachea, as it is in mammals. The right lobe of the thyroid is generally at the same level as the left. The left lies attached to the ventral surface of the left carotid artery, external to the trachea and esophagus, internal to the jugular vein, and anterior to the syrinx. The right lobe is similarly attached to the right jugular vein and lies externally to the carotid artery and trachea. The gland is a rich, reddish-brown color and is somewhat translucent (Forsyth, 1908).

### Functional Activity

A number of experiments and observations on colloid secretion by the thyroid during embryonic life attest to an early beginning of activity (Bradway, 1929; Sun, 1932). Sun (1932), after studying the function of various endocrine glands in the avian embryo, concluded that the thyroid was more significant during embryonic life than the other glands examined. It may well assume the functions later attributed to the parathyroid. If thyroxin is an essential catalyzer, regulating fundamental chemical reactions of the body, then it is conceivable that the production of this iodine-containing substance is an early requirement for normal development of the embryo. The presence of iodide at 12 and 15 days and its absence at 19 days (Blanquet, Stoll, Maraud, and Capot, 1953) necessitates further investigation as to its precise interpretation.

### Effect of Chemical Agents

The experimental use of synthetic or natural hormonal products generally produces either a stimulating or a depressing effect. The thyrotropic hormone (a thyroid-stimulating factor produced by the pituitary gland) causes the thyroid to become so active that it does not have the opportunity to store colloid. Removal of this factor decreases thyroid function, and colloid collects. Antithyroid compounds block thyroid activity and produce hypothyroidism. Thyroxin administration of thyroid implants overactivate the gland and produce hyperthyroidism.

**Thyrotropic hormone.** A complex relationship exists between certain glands of the endocrine system. The pituitary, or so-called master gland, exerts certain effects on various endocrine glands, among them the thyroid. The specific factor from the pituitary which stimulates the thyroid is the thyrotropic hormone. In the chick, this pituitary-thyroid relationship is probably established at about the eleventh day of incubation (Tixier, 1954). In the absence of the pituitary stimulation which is probably necessary for the later stages of normal thyroid development, the follicles of the thyroid develop only partially. Gaillard (1948) cultured thyroid glands from a 8.5-day chick in a hanging drop culture. Colloid vacuoles appeared, but normal follicles did not form. The same result follows hypophysec-



omy. In general, hypothyroidism follows the removal of the stimulating effect of the thyrotropic factor.

Conversely, the introduction of excess thyrotropic hormone is stimulating to thyroid growth. By injecting thyrotropic hormone from the anterior pituitary into chick embryos incubated 12 to 20 days, Woodside (1937*a*) obtained hyperthyroid specimens in which the thyroid had a high follicular epithelium and in which there was no colloid storage.

TABLE 21

The Effect of Various Antithyroid Drugs on the Developing Avian Embryo

Effect on the Embryo at Hatching Time	Investigator
Changes in thyroid gland:	
Increase in number and height of follicular cells	1a, 1b, 1c, 2, 3a, 3b, 3c, 5
Stored colloid vacuolated	3a, 3b, 3c
Amount of stored colloid decreased	1a, 1b, 1c, 2
Hyperemia of follicle cells	1a, 1b, 1c, 2
Beak and eyes unpigmented	3a, 3c
Limbs shortened	1a, 1b, 1c
Body weight decreased	1a, 1b, 1c, 5, 6
Hatching time generally delayed	1a, 1b, 1c, 3a, 3b, 3c, 4, 5, 6

1. Adams and Buss (1952)—used 14-day chick (*Gallus gallus*) embryo:  
1a—methyl thiouracil, injected 0.5 cc. of 0.2% solution.  
1b—thiourea, injected 0.5 cc. of 0.4% solution.  
1c—propyl thiouracil, injected 0.5 cc. of 0.2% solution.
2. Bennett and Adams (1952)—used 14-day chick (*Gallus gallus*) embryo; amphenone “B,” injected 0.5 cc. of 0.2% solution.
3. Mitskevitch (1949):  
3a—methyl thiouracil, injected 5 mg. into fresh pigeon (*Columba livia*) egg.  
3b—same, injected 2 mg. into 9- to 10-day thrush (*Turdus philomelos*) embryo.  
3c—same, injected 2 mg. into 9- to 10-day gull (*Larus ridibundus*) embryo.
4. Romanoff and Laufer (1956).
5. Wheeler and Hoffmann (1948*b*)—fed laying hen 0.02% thyroprotein containing 3.0% thyroxin; embryo examined at hatching.
6. Yushok (1950)—used 11-day chick (*Gallus gallus*) embryo; thiourea, injected 2.0 mg.

**Goitrogenic action of antithyroid compounds.** Drugs or compounds which suppress thyroid activity include a variety of substances, some of which are listed in Table 21, together with their effects. Such antithyroid drugs as thiourea, methyl thiouracil, and propyl thiouracil exhibit varying degrees of goitrogenic effect (Adams and Bull, 1949; Adams and Buss, 1951, 1952; Romanoff and Laufer, 1956). In general, the thyroid gland increases in weight after the administration of these drugs, because of the hypertrophy and hyperplasia of the follicular cells. The direct effect of



antithyroid drugs is to prevent the synthesis of thyroid hormone, and thus a hypothyroid state is produced. The resulting condition includes the vacuolization and release of colloid; a decrease in body growth, as evidenced by shortened limbs and decreased body weight; and a delay in hatching, with failure to retract the yolk sac.

Romanoff and Laufer (1956) demonstrated the influence of various doses of injected thiourea into fresh chicken eggs on length of incubation period and their hatchability. The results are shown in the accompanying table.

Thiourea Injected (mg.)	Incubation Period (days)	Hatchability (per cent)
0	21	93
2	24-28	75
3	24-28	15
5	26-31	0
10	29-31	0

They also showed that the eggs injected with thiourea (from 2 to 10 mg.) on the eleventh day produced on the twenty-first day of incubation a significant reduction in the weights of the entire embryo with a noticeable increase in the thyroid weight, especially from 2 mg. injection.

When thiouracil is fed to laying hens, the drug is transmitted to their eggs, since these hens produce chicks with enlarged thyroid glands (*Andrews and Schnetzler, 1945*).

Goitrogenic effects have been produced by various other experimental means. Substances such as thyroproteins have been administered orally, either to females, in which case their chicks are examined, or to day-old chicks. Enlargement of the thyroid gland results (*Andrews and Schnetzler, 1945; Wheeler and Hoffmann, 1948a, 1948b; McCartney and Shaffner, 1949a*). Similarly, injections of inorganic iodide produce goiter in chicks and prolong the incubation period (*Wheeler and Hoffmann, 1949*). Day-old chicks treated by either of these methods require 16 per cent less oxygen than control chicks (*McCartney and Shaffner, 1949a*). Feeding thyroxin to laying hens has been found to have no effect on the chicks (*McCartney and Shaffner, 1949b*), but the direct injection of thyroxin and iodinated casein into incubating eggs on the second, fourth, and sixth day of incubation reduced the size of the thyroid (*Booker and Sturkie, 1949*).

Thiourea-treated 11-day chick embryos grew normally until the fifteenth day of incubation but growth was delayed thereafter. Thus the thyroid significantly influences development of the chick embryo during the latter stages of incubation (*Yushok, 1950*). In fact, the liver and heart appeared to be target organs which were markedly affected by the thiourea treatment.

**Action and effects of thyroxin.** The effect of thyroxin has already been mentioned in connection with sexual development (see Chapter 10).



Its more general function of regulating metabolic rate was investigated by Beyer (1952), who compared metabolic rates of normal and thyroxine-treated chick embryos. The thyroid hormone was administered before the beginning of incubation. Treated embryos exhibited an increase in wet and dry weights; an increase in oxygen absorption during all stages of development, yet no significant increase in metabolic rate (calculated on the basis of wet and dry weights); and a decrease of 1.5 days in the length of the incubation period.

The administration of additional thyroid hormone by means of grafts produces a hyperthyroid condition. Willier (1924) grafted thyroids from adult chickens on the chorioallantoic membrane of chick embryos incubated 9 or 10 days. After the grafts had established vascular connections with the host, evidence of hyperthyroidism became apparent. The host exhibited a small and emaciated body; the segments of the legs and wings were shortened; internal organs were proportionately smaller than normal; and the amount of stored fat was less than in the controls.

#### *Effect of Other Experimental Methods*

The realm of experimental possibilities is virtually unlimited. The usual techniques involving endocrine glands include grafting and altering physical factors. Assay methods are useful in determining criteria for standardizing and measuring exact amounts of hormone.

**Adrenal grafts.** An example of the variety of interrelationships between the ductless glands is provided by the fact that some factor from the adrenal cortex seems to inhibit thyroid development. This effect is shown by grafting adrenal cortex taken from young rats on the chorioallantoic membrane of 8-day chick embryos (*Rokhlina and Studitskii, 1937*). Development is particularly inhibited between the twelfth and fourteenth days of incubation.

**Temperature effects.** Experimental work such as that of Carpenter (1945) has demonstrated the significance of temperature in the developmental pattern of the thyroid gland. Glands from chicks incubated 8 days were grown in tissue culture subjected to various temperatures. Exposure to 5° to 10° C. for 24 hours immediately following explantation did not retard the rate of thyroid differentiation. In some embryos returned to the normal incubating temperature, differentiation progressed more rapidly than in the untreated controls. Exposures longer than 24 hours are detrimental. Exposure without subsequent injury was possible only while the gland was an undifferentiated plate of cells. Low temperatures during the period of follicular arrangement prevented the gland from developing.

**Relation of thyroid to sexual development.** There is conflicting evidence concerning the effect of the thyroid on the development of the sexual organs. Greenwood and Chaudhuri (1928) concluded that sexual differ-



entiation proceeded independently of the level of thyroxin in the embryonic system. Thyroxin injected in 3-day incubated chick embryos caused hyperthyroidism but no unusual effects on the genital system. In adult birds, however, hyperthyroidism is known to alter normal plumage types.

## THE PARATHYROID GLAND

The parathyroid glands are four small bodies of internal secretion which arise in close association with the thymus gland, since both are probably derived from the third and fourth pharyngeal pouches. Thus they are endodermal in origin. The parathyroids are usually thought of as being closely associated with the thyroid gland, for they come to lie directly caudad to the thyroid. The parathyroids can be characterized as masses of epithelial cell cords interspersed by numerous small blood vessels.

### *Morphogenesis of the Parathyroids*

The parathyroids are generally believed to originate from pharyngeal pouches. Venzke (1947), however, claimed that the parathyroids of the duck (*Anas platyrhynchos*) are separated from the thyroid lobe by an invasion of connective tissue after 148 hours of incubation. After they detach, the primordia move posteriorly from the thyroid lobes and are then divided into anterior and posterior lobes by another invasion of connective tissue. On the other hand, the work of Verdun (1898) and the cell-tagging experiments of Schrier and Hamilton (1952) show that the parathyroids are derived from the third and fourth pharyngeal pouches in much the same manner as the thymus gland. Verdun stated that the ventral parts of these pouches give rise to the parathyroids, which then migrate to a position immediately posterior to the thyroids. This finding was confirmed by the observation that marking the third and fourth visceral clefts with carbon resulted in the appearance of carbon in the parathyroid and thymus glands (Schrier and Hamilton, 1952).

Observations on the developing gland show that it has a cordlike structure similar to that of the thyroid. Sun (1932) first noted the typical structure in the 8-day chick. The parathyroid differs from the thyroid in that the former consists of a thicker cord and lacks vesicles. Groups of cells are separated by connective tissue. Since the embryonic character of the gland persists throughout incubation, Sun (1932) concluded that the gland probably does not function in the embryo chick.

The histological study of the developing gland made by Venzke (1947) showed that the anterior lobe is at first crescent-shaped and surrounded by mesenchymal cells. The smaller lobe was found to be 50 microns posterior to the anterior lobe (cf. Fig. 328-B<sub>1</sub>). The cells of the smaller lobe stain deeply basophilic with hematoxylin. In the 9.5-day chick, the anterior lobe



is semicircular and gives off branches of cellular cords. The mesenchyme between the cords contains capillaries. As development continues, the cellular cords increase in size, show much mitotic activity, become more closely packed, and are surrounded by increased numbers of blood vessels. At 13 days, two types of cells are present. First, there are large, clear cells with slightly chromatic nuclei; these cells subsequently decrease in number. Cells of the second type possess highly chromatic nuclei and acidophilic cytoplasm. These cells predominate in the glands, even after the appearance of a third cell type. The latter, which appears after 17 days, has granular acidophilic cytoplasm and a basophilic nucleus. Toward hatching, the glandular tissue is extremely compact, and the size of the sinusoidal capillaries is reduced. The cell cords are now two cells thick. Both lobes of the parathyroid are in close proximity and reticular fibers connect the two lobes.

### *Function*

It is well established that the role of the adult parathyroid is the regulation of calcium and phosphorus metabolism. This has been demonstrated by parathyroidectomy, which results in tetanus, convulsions, and death, unless calcium and magnesium ions are administered, in which case improvement is rapid. Little has been done on the parathyroids of the avian embryo, and, according to Sun (1932), the gland is believed not to function in the embryo. There is no evidence that the functional status of the parathyroids is conditioned by any of the hypophyseal hormones.

## THE THYMUS GLAND

The thymus gland is included here because of its close association with the other endocrine glands which are also pharyngeal derivatives. However, no one has yet demonstrated with certainty that the thymus is actually a gland of internal secretion. It has long been known to arise from the third and fourth pharyngeal pouches, and it is highly lymphoid in nature. It is not known whether it functions in hormone secretion in the embryo bird.

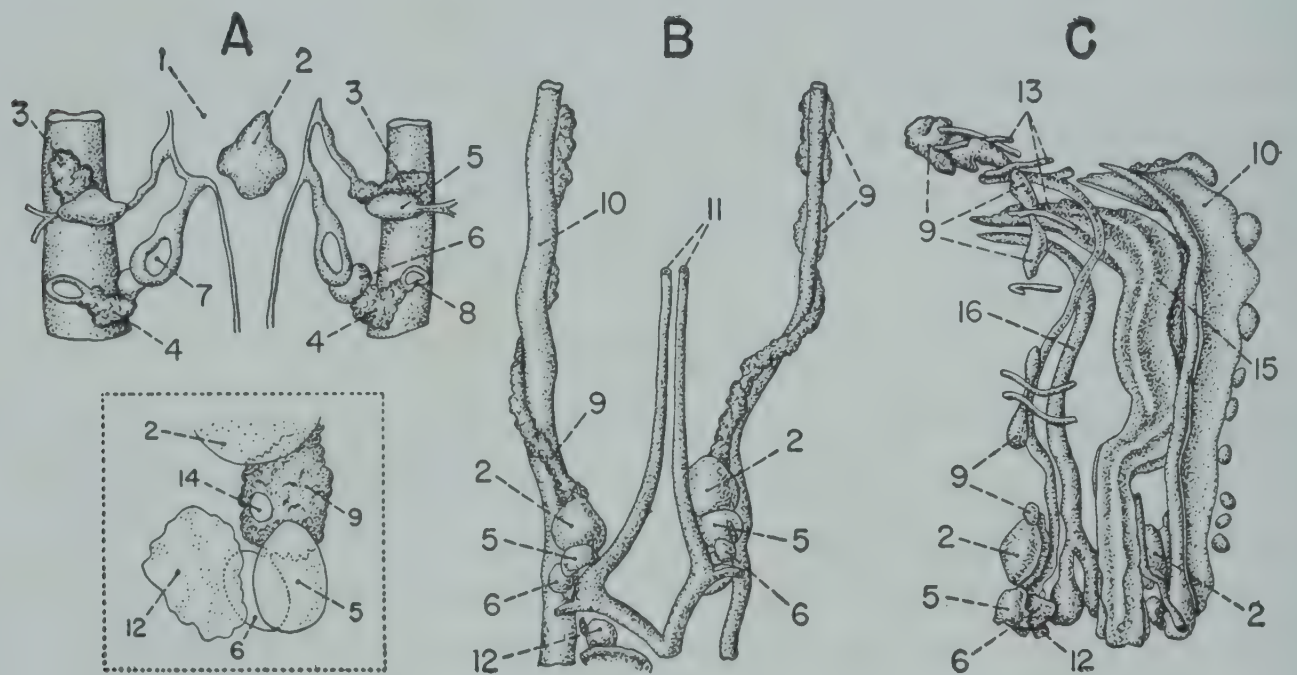
### *Thymus Development*

The thymus, because of its conspicuous size and location, has long been observed and studied. One of the first accounts is that of Verdun (1898), in which it is stated that the gland is the derivative of the third and fourth pharyngeal pouches. This view has been corroborated by more recent workers (Venzke, 1942, 1952; Schrier and Hamilton, 1952; Hammond, 1954). Hammond (1954) states that branchial ectoderm contributes largely, if not entirely, to the epithelial component of the gland, though earlier



authors, with the exception of Kastschenko (1887), believe the thymus to be of endodermal origin (Mall, 1887; Verdun, 1898; Hamilton, 1913; Helgesson, 1913; Johnson, 1918a; Schrier and Hamilton, 1952). The third pouch provides the greater portion of the gland. The dorsal walls of the fourth pouch contribute to the thymus.

The thymus is first visible as a rudiment in the 5.1 mm. sparrow (*Passer domesticus*) embryo (Helgesson, 1913), in the 5-day chick embryo (Venzke, 1952), and in the 3-day duck (*Anas platyrhynchos*) embryo



**Fig. 330.** The developmental relationships of the thymus gland in the avian embryo. (Redrawn with modifications A, B, and *Insert*, after Verdun, 1898; C, after Helgesson, 1913.)

A, chick embryo of 164 hours, reconstruction of thymic region at level of third pharyngeal slit ( $\times 15$ ); B, 240-hour chick embryo in which the thymus is an elongated lobulated gland twisting around the internal jugular vein ( $\times 10$ ); C, reconstruction of 14-mm. sparrow embryo (*Passer domesticus*) showing the left side which has four thymus lobes ( $\times 20$ ).

*Insert*: definitive relationships of thymus, thyroid, and parathyroid glands in adult chicken ( $\times 5$ ).

1, pharynx; 2, thyroid; 3, thymus III primordium; 4, thymus IV primordium; 5, parathyroid III; 6, parathyroid IV; 7, cavity of postbranchial body; 8, fourth endodermal pouch; 9, thymic lobe; 10, internal jugular vein; 11, carotid artery; 12, postbranchial body; 13, cervical nerve; 14, carotid gland; 15, esophagus; 16, trachea.

(Hamilton, 1913). In the sparrow (*Passer domesticus*), it is described as a hollow cellular primordium which begins to elongate cranially and is pressed around the parathyroid both laterally and ventrally. In the 6.7-mm. sparrow, the thymus anlage has a lumen caudally. The gland elongates anteriorly, and the part which lies over the hypoglossal arch is a solid mass of tissue. At the 8.7-mm. stage, the thymus consists of two distinct parts (Fig. 330-A). The cranial half is budlike and round and lies anterior to the hypoglossus nerve; the part posterior to the nerve is elongate and cordlike. The



lower end is continuous with the parathyroid. By the 10- to 12-mm. stage, the thymus has six lobes on either side of the embryo, the upper being the largest and corresponding to the original anterior half. The rest are derived from the earlier small posterior lobe. The lobes may appear as a continuous or broken chain twisting around the jugular vein (Fig. 330-B). At 14 mm. the embryo has eleven distinct lobes on the right side and only four on the left (*Helgesson, 1913*).

Development is similar in the chick. At first the thymus primordium is a mass of epithelial cells which elongates to form an epithelial cord extending along the jugular vein. This tissue is soon joined by the cells of the fourth pouch which come from its dorsal wall and intermediate part. At the 5.5-day stage, the third and fourth pouches close off from the pharynx and then appear as masses of epithelium adjacent to the jugular vein. The dorsal portion of the fourth pouch fuses with the ventral portion of the epithelium of the third pouch. At the end of the seventh day and thereafter, the elongate thymus is syncytial and appears reticular (Fig. 330-C) because of the finely branched protoplasmic threads of which it consists (*Venzke, 1952*).

There has been some question as to whether the thymus always comes from the ventral and dorsal parts of the third pouch, or whether the thyroid, the ectoderm of the visceral groove, and adjoining cervical sinus may not also contribute to the gland. Marking the cells of the pharyngeal pouches with carbon (*Schrier and Hamilton, 1952*) showed that the third and fourth pouches were the principal contributors to the thymus.

The lymphatic nature of the thymus is well known. *Venzke (1952)* noted that the thymus of the 7-day duck (*Anas platyrhynchos*) embryo is a syncytial mass closely invested by lymphocyte-containing mesenchyme, but that there are no lymphocytes in the gland itself. After 8.5 days of incubation, these cells have migrated into the thymus tissue. In a few more hours, the epithelial cells produce lobules of tissue, so that thymus tissue extends irregularly from the head to thoracic regions. The greatest concentrations of lymphocytes occur in the region of the head. At this time, the epithelial cells of the thymus have light-staining cytoplasm, and their nuclei are oval, with very little chromatin material. The gland is not yet vascularized. The lymphocytes in the mesenchyme are of both large and small types. They first appear in the thymus at the 10-day stage. Lobes continue to be formed and lymphocytes gather around the lobes, but blood vessels are still outside the thymus. At the end of the eleventh day the first blood vessels enter the thymus, and the blood supply increases rapidly thereafter. Large lymphocytes concentrate around the blood vessels, while small lymphocytes are the general small thymic cells of the gland.

In the 12-day embryo, the lobules of the gland have a densely packed periphery, whereas the central regions are looser and contain the concentra-



tions of blood vessels. Thus there are distinct cortical and medullary zones. Epithelial cells in the medulla may form clumps; Venzke suggested that these are the rudiments of Hassall's corpuscles, which in the adult are clusters of degenerating, acidophil cells. In the 14.5-day chick, the corpuscles are developing further. Eosinophil cells appear in great numbers after about 16 days 16 hours of incubation, and, at the end of the seventeenth day of incubation, the thymus is well defined into zones. Small thymic cells form a dense cortex. From a connective tissue capsule, ingrowths divide the gland into septa. These lobules are at first separated by loose cellular connective tissue, but as they grow, the connective stroma is compressed by thin septa. The stroma contains connective tissue cells, lymphocytes, eosinophils, and large blood vessels. The original large epithelial cells become reticular cells, are syncytial in nature, and comprise the medulla. Hassall's corpuscles are present in the medulla and appear as groups of three or four hypertrophied reticular cells. At the 19-day stage, these corpuscles are acidophilic and range from 30 to 40 microns in diameter (Venzke, 1952).

**Gross aspects.** The thymus weighs less in female than in male chick embryos, although there is considerable individual variation (Venzke, 1943). A noticeable trend in thymus weight is its progressive increase from the tenth day of incubation until just before hatching. In a comparison of thymic weight with body weight, Dmitrieva (1939) found that the thymus develops slowly in proportion to the rest of the body from the tenth to the sixteenth incubation day as shown in the accompanying table.

Age of Embryo (days)	Body Weight (gm.)	Thymus Weight (mg.)
10	1.72	3.421
12	2.54	4.657
14	7.68	7.026
16	12.60	8.690
18	20.24	26.177
21	35.89	37.632

After the sixteenth day, the thymus increases greatly in weight, and at the same time metabolism changes are great and mortality may be high. At hatching an abrupt decrease in thymic weight in both sexes is noticeable. The percentage decrease in respect to body weight continues, except for a period of several months when the thymus may actually weigh more; but this is eventually followed by the regression of weight and size which characteristically occurs in adults.

On the day after hatching, the thymus gland has been found to weigh 0.101 grams or 0.312 per cent of the body weight (Latimer, 1924). The thymus is one of the largest glands in embryonic and young chicks.



### *Function of the Thymus*

According to the work of Venzke (1952), the development of the thymus seems to be independent of the influence of other endocrine glands.

Thymectomy can be achieved with a complete removal of the thymic tissue. No specific endocrine function has been attributed to the thymus gland. It does not seem to exert any control of calcium metabolism (Maughan, 1938).

Toward the end of incubation the thymus is one of the organs of the body in which hematopoietic activity takes place. This continues into the postembryonic period, probably until thymic regression takes place.

**The Definitive Thymus.** In a hatched bird, the thymus follows the course of the jugular vein and the vagus nerve through the upper thorax aperture. It is an elongated, lobulated gland whose enlarged cranial end lies in the vicinity of the third cervical segment and extends downward to the thoracic cavity. It usually has fourteen lobes, seven on each side. Although they extend to the thoracic cavity, they are not located in it. The thyroids and parathyroids are caudal to the thymus (Maughan, 1938).

There is no indication of a possible endocrine function of the thymus in the embryo. After hatching, the thymus is known to influence growth and development. It also seems to increase resistance to avitaminoses, bacteria, and malignant tumors (Dmitrieva, 1939).

## THE ADRENAL GLAND

The adrenal gland has long been recognized as an endocrine organ which is essential to life and which controls certain aspects of normal function. In birds, it begins development and function early in the incubation period. The avian adrenal gland, like that of most vertebrates, has a double origin. That is, the two components, cortex and medulla, are derived independently of one another, even coming from different germ layers. The cortical portion is the first to be differentiated. These cells bud off from the peritoneal epithelium after the first few days of incubation. Medullary or chromaffin cells appear several days later, arising from the primordium of the sympathetic nervous system, which is ectodermal in origin (see Chapter 4).

The structure of the avian suprarenal gland is similar to that of the amphibian and reptilian types in that the cortical and medullary cords are irregularly intermingled. This is quite unlike the situation in mammals, where the cortex forms a rather distinct capsule around the medulla. In birds, as in other vertebrates, adrenal function is vital to life and growth even from embryonic stages. The fact that there are two entirely different components of the gland results in a dual function, for the adrenal acts both as a gland and as a part of the sympathetic nervous system.



### Early Development

Much diversity of opinion as to the origin of cortical and medullary cells has accompanied the accumulation of knowledge concerning the adrenal. Both cellular components differ widely in their mode of origin.

#### *The Adrenal Cortex*

The suprarenal cortex develops independently from the other component of the adrenal, the medulla. Cortical cells are generally believed to arise in the peritoneal epithelium. After migrating dorsally, the cells form solid bodies having a cordlike structure.

**Theories of origin.** The cortex has been shown to be ectodermal in origin. This relationship was observed by Janošik (1883), Mihalkovics (1885), Valenti (1889), Fusari (1893), and Goormaghtigh (1921). However, several workers have maintained the belief that the mesoderm gives rise to cortical cells; this opinion was expressed by His (1868), Waldeyer (1870), and Brunn (1872). In addition, Weldon (1885) claimed that the cortex is derived from the mesonephros, whereas Semon (1890) and Rabl (1891) stated that its source is the pronephros.

**Morphogenesis of cortical cells.** Adrenal development begins with the appearance of the cortical substance. The first cells arise as a thickening of the peritoneal epithelium, located ventral and medial to the mesonephros, ventral to the abdominal aorta, and dorsal to the hind-gut. The primordial cortex cells are formed in the chick on about the fourth day of incubation (Brunn, 1872; Valenti, 1889; Minervini, 1904; Hays, 1914; Willier, 1930; Venzke, 1942), and on the third day in the pigeon, *Columba livia* (Minervini, 1904). Cortical cells in the chick embryo were first seen at 78 hours by Rabl (1891), Fusari (1893), and Soulié (1903), although early workers did not observe these until the seventh day (Gray, 1852) and the fifteenth day (Remak, 1855).

By taking grafts of the portion of the Wolffian body in which the potential suprarenal cells are localized, Willier (1930) was able to get independent development of the gland merely by culturing the potential cells on chick chorioallantois. Cells of the chick's suprarenal cortical primordium were localized in the Wolffian body as early as the ninety-sixth hour. They were in the thickened coelomic epithelium at the medioventral aspect of the anterior end of the mesonephros.

Soon after their formation, the prospective cortical cells leave the epithelium and move dorsally (Fig. 331-A and B), becoming larger and more circular than the peritoneal cells. The nuclei of the new cells are larger, stain less deeply, and are less granular than those of the peritoneum.

During the early stage of development, the cortical substance increases very rapidly, and by the one hundred-fifth hour (Hays, 1914) the cortical



cells have formed paired solid bodies on each side of the base of the mesentery (Fig. 331-C).

Soulié (1903) describes cords of 30 to 50 microns in length located in the connective tissue at this stage. The cortical cell concentrations lie mesial to the ventral side of the mesonephros, remaining connected to the

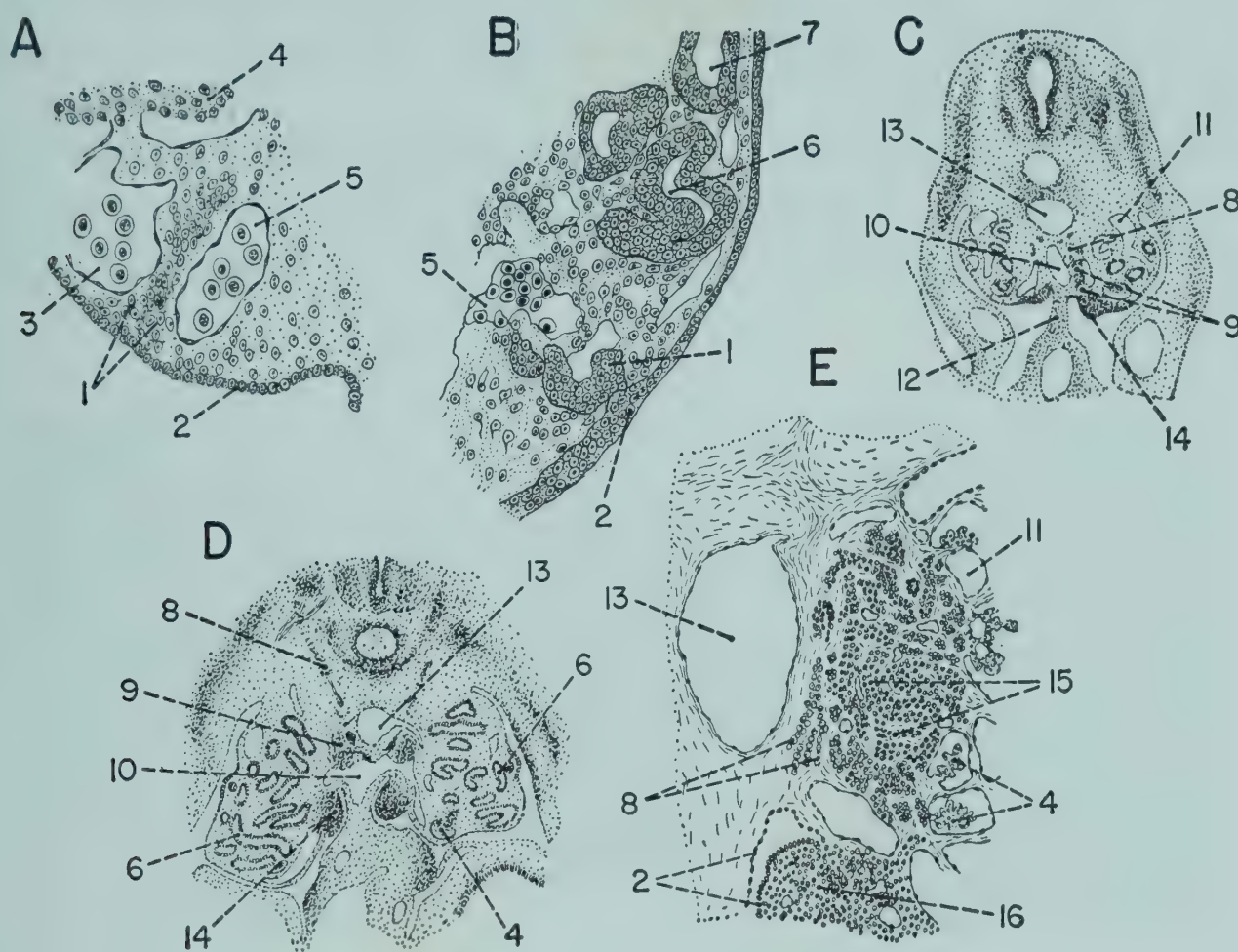


Fig. 331. Development of the adrenal gland in the parakeet (*Melopsittacus undulatus*) and chick (*Gallus gallus*) embryo. (Redrawn with modifications A, C, D, and E, after Soulié, 1903; B, after Rabl, 1891.)

A, 5.2-mm. parakeet embryo section through region of proliferation of primordial cortical cells ( $\times 250$ ); B, 79-hour chick, showing adrenal cortical cells migrating dorsally from the peritoneal epithelium ( $\times 250$ ); C, 5.5-mm. parakeet, showing relationship of suprarenal cells to other structures and cortical cells migrating dorsally to meet sympathetic ganglia ( $\times 30$ ); D, 8.5-mm. parakeet embryo, transverse section through dorsal portion of Wolffian body ( $\times 10$ ); E, 15-mm. parakeet, transverse section of adrenal body ( $\times 40$ ).

1, primordial cells of adrenal cortex; 2, coelomic epithelium; 3, vein of Wolffian body; 4, mesonephric glomerulus; 5, blood sinus; 6, mesonephric tubule; 7, Wolffian duct; 8, sympathetic ganglion; 9, adrenal cortical cells; 10, venous trunk connecting mesonephric veins; 11, posterior cardinal vein; 12, mesentery; 13, aorta; 14, gonad primordium; 15, adrenal cortical cord; 16, gonad.

peritoneal epithelium by a strand of cells (Hays, 1914) which indicates the path of migrating cells en route to the level of the aorta.

On the chick's fifth day, the cortical cells detach from the peritoneum and the migration to the dorsal position is nearly complete (Fig. 331-D). The cells are arranged in scattered groups in the mesenchyme between the mesonephros and the aorta (Weldon, 1885; Fusari, 1893; Soulié, 1903;



Minervini, 1904; Brezzi, 1940). Their staining properties readily identify them. The cortical cells fill nearly the entire region dorsal to the subcardinal veins, lateral and ventral to the aorta, and mesial and ventral to the post-cardinal veins (Fig. 331-E). Posteriorly, they extend to about the level of the anastomosis of the subcardinal veins in the median line (see Chapter 8). The time at which the primordia reach the efferent vessels and the posterior cardinal veins and come into intimate contact with them is about 94 hours (Soulié, 1903).

The mass of cortical substance increases steadily by the collection of the cells into large groups and by mitotic division of the cells in these groups. During the sixth day, the cells are even more closely grouped in large oval

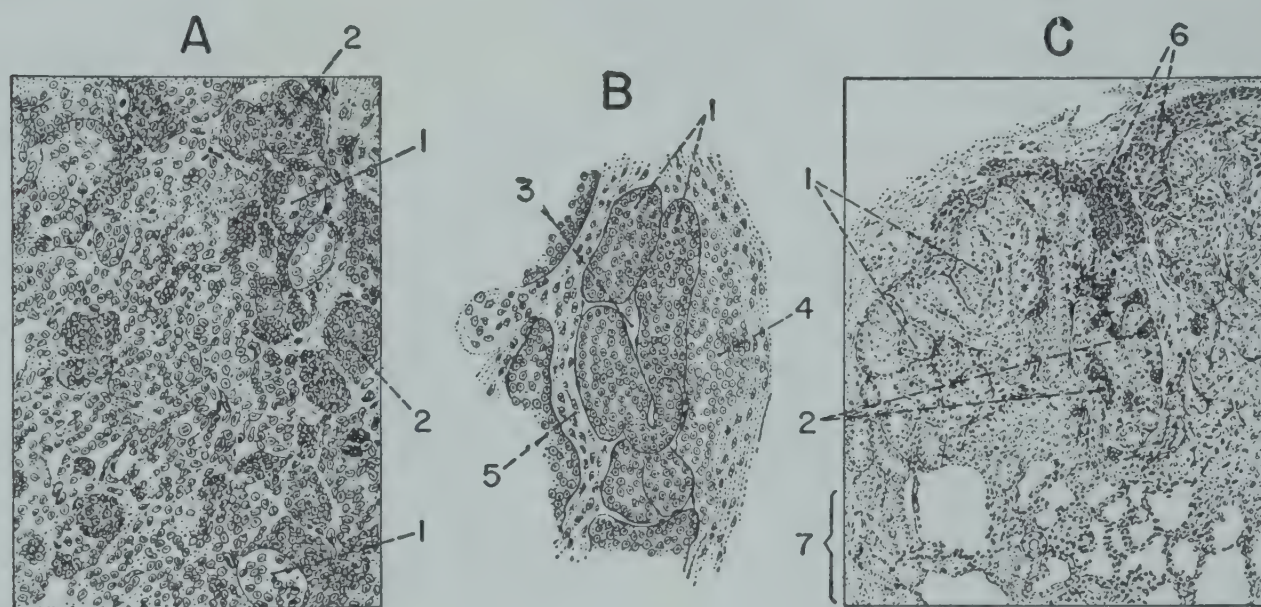


Fig. 332. Histology of the adrenal gland of the chick embryo. (Redrawn with modifications A and C, after Willier, 1930; B, after Rabl, 1891.)

A, suprarrenal tissue differentiated in a graft of a Wolffian body isolated from a 4-day embryo and grown 9 days ( $\times 140$ ); B, dorsal part of adrenal gland of 16-day male embryo ( $\times 140$ ); C, suprarrenal graft grown from Wolffian body for 9 days, taken from 4-day embryo ( $\times 60$ ).

1, cortical cord; 2, medullary cord; 3, blood sinus; 4, connective tissue; 5, intercortical tissue; 6, sympathetic cells; 7, region of Wolffian body.

masses on each side of the aorta. They lose their original outlines, but their nuclei retain their shape, are more granular, and still contain mitotic figures. A thin layer of mesenchyme separates the cortical cell bodies from the mesonephros.

**Formation of cortical cords.** During the seventh day the mass of cortical substance is even larger than before and the cells are arranged in cords and have a roughly hexagonal shape. Cortical cords in grafts (Willier, 1930) are quite normal in development (Fig. 332-A and C), being cylindrical in form, and having light-staining cells radiating from the center; but the cords lack a central lumen. The nuclei stain darker and continue to exhibit many mitotic figures. The cortical mass is roughly circular and is in contact with the ventral angle of the mesonephros. The growth of con-



nective tissue around the cortical cells is the beginning of the tissue capsule which forms later. Some of the fibers penetrate the gland, going in between the cords (Fig. 332-B). At the 8-day stage, after continued growth of the cortical tissue, the mass is twice as large in cross sectional area as the aorta (Hays, 1914). At this same stage blood cells appear between the cortical cell cords, and vascularization increases during the next 24 hours.

### *The Adrenal Medulla*

The second principal component of the adrenal gland, the chromaffin tissue, has a developmental history quite different from that of the cortex. In fact, the source of the so-called mesodermal substance is ectodermal. Its source is known to be the anlagen of the prevertebral sympathetic plexuses (Fusari, 1893; Soulié, 1903; Hays, 1914; Rau and Johnson, 1923). The work of Willier (1930) suggests that chromaffin cells may be derived from mesenchymal cells, cortical cells, or cells of the primary sympathetic trunk, most likely the latter (see Chapter 4). The secondary sympathetic chain may also make supplementary contributions (Dawson, 1953).

**Appearance of medullary cells.** Medullary cells were first seen in the chick's suprarenal complex at 5 days 7 hours of incubation with the aid of the silver impregnation method of blackening medullary cells (Dawson, 1949, 1953). Histochemical evidence of differentiation of the elements of cortical cords can be obtained 24 hours later, but these positive reactions are not uniform or present in all cords until the eleventh or twelfth day.

On the fifth day, there is no apparent association between the chromaffin cells and the cortical cells. While the latter are arranging themselves into large masses, the former appear as large oval cells migrating ventrally from the sympathetic trunks on each side of the aorta (Harris, 1928). They migrate singly, and those not entering the suprarenal form the prevertebral sympathetic plexuses. The sympathetic cells migrate in a path between the aorta and the groups of cortical cells (cf. Fig. 331-D). They are easily distinguishable from the latter by the larger size and deeper stain of the chromaffin cells. The grafting experiments performed by Willier (1930) clearly demonstrated that the primary sympathetic cells contribute to the formation of the medullary cords and that secondary sympathetic cells are not essential to suprarenal formation. The work of Venzke (1942) indicates that secondary sympathetic trunks probably contribute to medullary cords after the sixth day of incubation.

**Association with cortical elements.** The first evidence of connection between cortical and medullary components has been variously put at 90 to 120 hours (Fusari, 1893), 102 hours (Goormaghtigh, 1921), and 130 hours (Hays, 1914; Dawson, 1953). At the time when the two components become associated, the cortical cells are in large compact masses of definite outline. Some of the chromaffin cells migrating from the sympathetic trunks



turn off ventrally and enter the adrenal masses or become attached to the surface of the cell groups (cf. Fig. 332-C). There is no apparent morphological difference between the cells which penetrate the adrenal and those which continue to migrate to form prevertebral sympathetic plexuses. When more chromaffin cells have entered the gland, they collect in groups of two or three. At 165 hours, ganglionic as well as nonganglionic sympathetic cells from primary and secondary trunks have migrated into the adrenal tissue (Venzke, 1942).

**Further growth.** The chromaffin cells begin to differentiate after they have penetrated the suprarenal. On about the seventh day (Hays, 1914) they change from large, circular cells with round, clear nuclei to irregular-shaped, small cells with oval, granular nuclei. Their characteristic feature is still their strong affinity for stains.

The invading sympathetic cells tend to arrange themselves in cords between the cortical substance. During the period of active invasion into the gland, the mesenchyme around the gland is completely filled with the sympathetic cells and the cells enter the gland from all directions.

By the ninth day of incubation, the sympathetic cells around the periphery of the adrenal gland have disappeared from the mesenchyme. The chromaffin cells within the gland increase in number, still arranged in cords or columns, which later break up. The cells then cluster in groups around the venous blood vessels (Hays, 1914). In grafts, Willier (1930) observed the dark-staining medullary cells forming strands or clustering in groups, never quite forming distinct cords. These cell clusters are closely applied to cortical cords and may even be intimately related to them. Mitosis is rare in medullary cells. The distribution of these cells among cortical cords is variable in grafts. Usually the medullary cells are at the periphery of surface of the suprarenal. Medullary cords may penetrate the gland and distribute uniformly throughout.

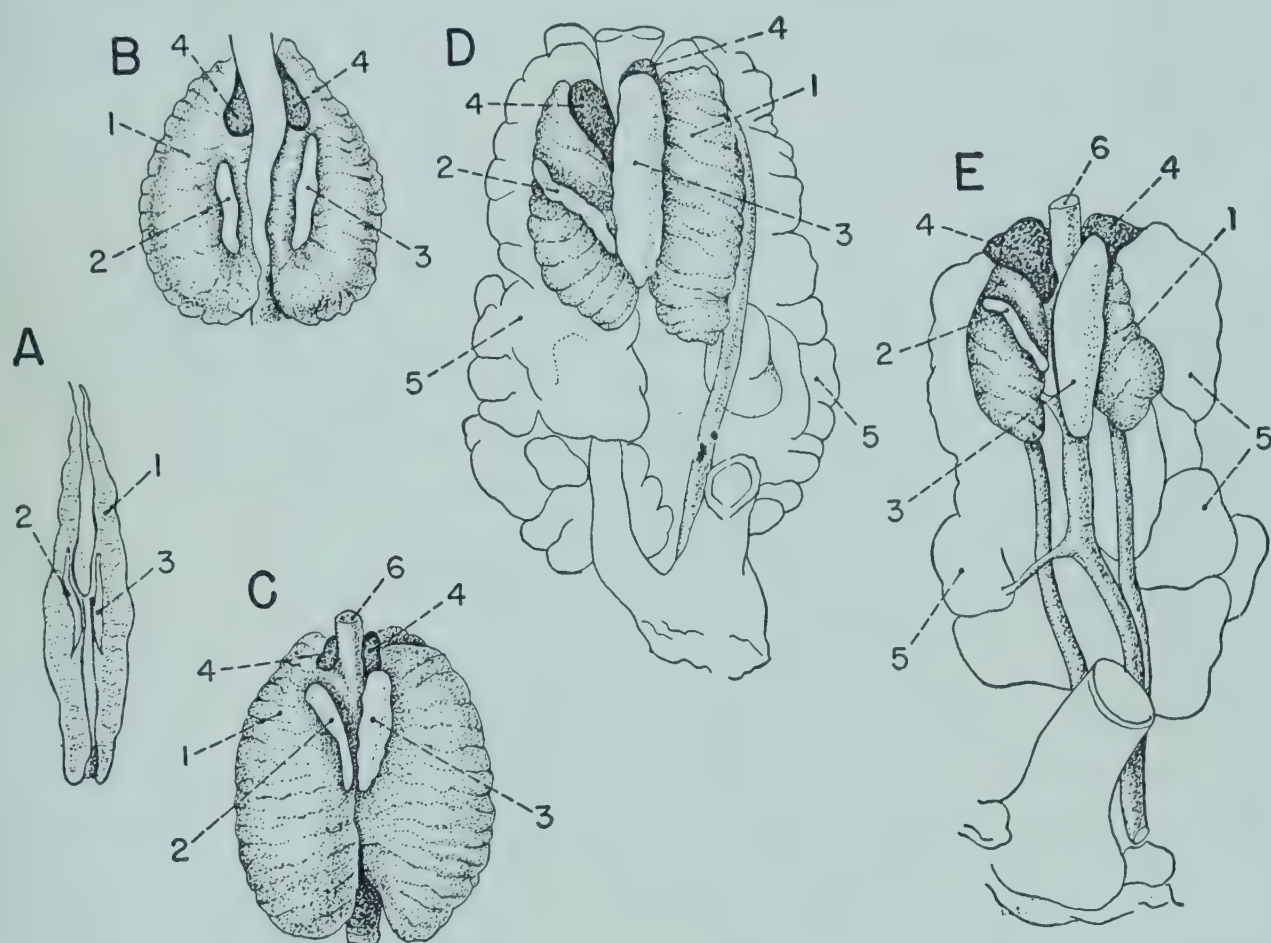
### Later Development

Development in the chick embryo after the ninth day is largely concerned with the growth of the cortical and chromaffin tissue. The glands increase in volume and vascularity throughout embryogeny (Fig. 333). Venous sinusoids form a network throughout the entire gland. They appear molded on the surface of the cords, without intervening mesenchyme. The blood spaces are larger in the center of the gland and are of capillary size near the periphery. The cortical cells are in irregular columns which pass around these blood vessels and seem to form the foundation for all other elements of the gland. The chromaffin cells are found in groups of all sizes, each group in contact with a venous blood vessel. In grafts which closely approximate the normal, the center of the adrenal is sinusoidal, the bulk of the gland consists of a network of cortical cords, and a large blood



vessel enters the suprarenal at one side, giving this portion the appearance of a hilum. In these grafts, both ganglionic and nonganglionic sympathetic cells are contiguous with the surface of the suprarenal.

The connective tissue capsule which started development on the seventh day has formed a dense capsule around the gland by the seventeenth day of incubation (*Hays, 1914*). Only very small fibers penetrate the gland and these are confined to the spaces between the cortical cords.



**Fig. 333.** Gross appearance showing semidiagrammatically the suprarenal glands and their relation to the urogenital system at different ages of the chick embryo. (Redrawn with modifications after Gray, 1852.)

A, elongated mesonephros and ovaries in 5-day embryo, before appearance of suprarenals; B, suprarenals present at upper, median margins of Wolffian bodies as seen in 7-day embryo; C, 10-day embryo; D, 14-day embryo; E, 18-day embryo.

1, mesonephros; 2, right ovary; 3, left ovary; 4, suprarenal gland; 5, metanephros; 6, dorsal aorta.

**Symmetry.** The laterality of the suprarenal is determined at an early age, as indicated by the differences in growth capacities of the right and left primordia. Willier (1930) noticed that whenever one suprarenal was smaller than the other, the right gland was the smaller in seven cases out of ten. Of thirteen grafts in which the suprarenal failed to develop, eleven were taken from the right Wolffian body and only two were grafts of the left mesonephros. Goormaghtigh (1921) noted that the right normal cortical primordium is usually smaller than the left. This asymmetry also occurred more often in females than in males, the smaller gland being associated with the small right female gonad.



**Definitive condition.** In the definitive condition, the adrenal glands lie just anterior to the bifurcation of the postcaval vein, one on each side of the median line. Anteriorly they extend to the inferior angle of the lungs. The right gland is roughly triangular (Fig. 333-E), while the left is oval in outline (Hays, 1914); or they may both be oval, elongate, or pear-shaped (Minervini, 1904). The internal arrangement of the cortical and chromaffin substance is similar to that observed in the embryo at the end of incubation, when cortical and medullary substances are thoroughly intermingled, generally with medullary cords scattered in the reticulum of cortical cords.

### Adrenal Function

The active agent in the adrenal gland was tested for potency by noticing its mydriatic (dilating) action on the enucleated eye of the frog. Positive action was found in 8-, 9-, and 10-day chick embryos (Lutz and Case, 1925). This effect was given by the adrenal tissue alone, although many related tissues were tested. The adrenal, therefore, seems capable of functioning on the eighth day. This time corresponds to the period when medullary cords begin to penetrate the cortical material.

Sun (1932) found that epinephrine is present in the adrenal glands of the chick embryo at the 9.5-day stage and that it increases twenty-six times in amount between this time and the hatching date. The intestinal strip test is one method of determining adrenal activity. The method depends on the presence in the adrenal of an active secretion which will inhibit normal rhythmic contraction and tonus in isolated gut. This effect was achieved by Hogben and Crew (1923) when 16-day incubated chick adrenals were used. These workers demonstrated chromophil tissue in 15-day chick embryo adrenals. By chromophil tissue, they were referring to the chrome-staining tissue of the medulla. The chromic reaction gave evidence of the appearance of adrenalin in 8-day incubated chick embryos in the intestinal strip experiments of Okuda (1928).

The adrenal function in adult birds as well as in other vertebrates has long been the subject of extensive investigation. Acid-base balance and glucose regulation are attributed to the medulla, while the cortex hormone apparently affects resorption of sodium and potassium. Whether these same processes are directed by similar hormones in the avian embryo is a matter which warrants investigation.

### THE PITUITARY GLAND

The pituitary gland, or hypophysis, as it is often called, is perhaps the most important of the endocrine glands in the adult bird. In addition to secreting hormonal substances which are essential to normal metabolism, it



acts as a coordinator of other endocrine organs. Even though the role of the hypophysis in the avian embryo is not clearly understood, the gland has been extensively studied, partly because of its later importance and also because of its relationship with the developing nervous system.

The pituitary gland is a small, lobed organ located at the base of the brain directly behind the optic chiasma in a concavity (sella turcica) of the sphenoid bone. The gland is a compound organ which is partly epithelial and partly neural (thus wholly ectodermal) in origin.

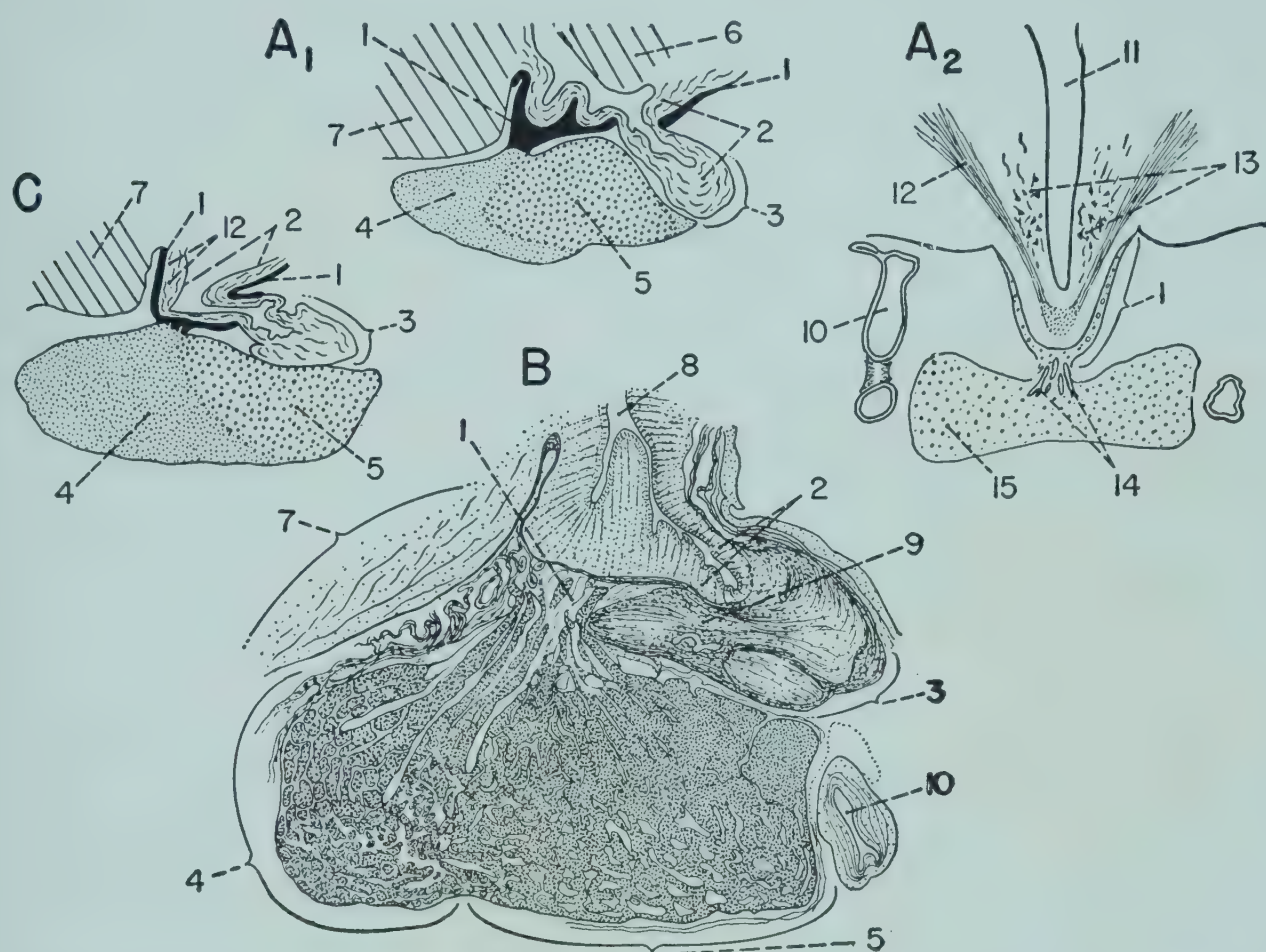


Fig. 334. Structural relationships in the definitive hypophysis of chicken, *Gallus gallus*, duck, *Anas platyrhynchos*, and pigeon, *Columba livia*. (Redrawn with modifications A<sub>1</sub> and C, after Rahn and Painter, 1941; A<sub>2</sub>, after Drager, 1945; B, after Economo, 1899.)

A<sub>1</sub>, diagrammatic sagittal section of chick hypophysis ( $\times 10$ ); A<sub>2</sub>, diagrammatic frontal section of chicken hypophysis ( $\times 10$ ); B, sagittal section of post-hatched pigeon hypophysis ( $\times 30$ ); C, diagrammatic sagittal section of duck hypophysis ( $\times 9$ ).

1, pars tuberalis; 2, infundibular process; 3, pars nervosa; 4, cephalic lobe of pars anterior; 5, caudal lobe of pars anterior; 6, brain tissue; 7, optic chiasma; 8, infundibulum; 9, connective tissue; 10, carotid artery; 11, third ventricle of brain; 12, hypothalamico-hypophyseal fasciculus; 13, nuclear cell masses; 14, vascular channels; 15, pars distalis.

The epithelial hypophysis (pars buccalis) is derived from the oral epithelium. It has its origin in Rathke's pouch, a hollow diverticulum which extends upward from the stomodaeum to the floor of the diencephalon in the region of the infundibulum. Meanwhile, an outpocketing of the infundibulum forms the primordium of the posterior pituitary lobe (pars nervosa), also termed processus infundibuli.

The pituitary lobes derived from Rathke's pouch are grouped under the



term adenohypophysis. The larger lobe, the pars distalis, is ventrally located. The smaller pars tuberalis is dorsal to the cephalic portion of the pars distalis (Fig. 334-A<sub>1</sub>). The pars tuberalis is a thin epithelial plate of cells which is formed by the fusion of two outgrowths from the pars distalis. The class *Aves* is unique in lacking a pars intermedia between these two lobes. The hypophyseal parts derived from the diencephalon constitute the neurohypophysis. The principal structure is the neural lobe (infundibular process), which is dorsal to the caudal portion of the large, ventral pars distalis (Fig. 334-B and C). In addition, the neurohypophysis includes the infundibular stem which connects the neural lobe to the pars distalis. The infundibular stem, however, is prominent only during early embryonic stages.

The pituitary gland is encapsulated by the dura mater. The vascularization of the anterior lobe is extensive. The innervation of the gland has not been completely determined; fibers are received from the hypothalamic portion of the brain and supply principally the pars nervosa. The cytology of the gland is concerned with the distribution and variation in the three principal types of cells: chromophobes, acidophils, and basophils. The gland assumes some of its significant functional roles during the latter half of the incubation period.

### Developmental Morphology

In tracing the development of the pituitary, it is logical to start with the stomodaeal derivatives, since Rathke's pouch appears first, after the close of the first day of incubation. Subsequently, the infundibular contribution, the pars nervosa and its stalk, join the anterior lobe.

The development of Rathke's pouch and the early epithelial lobe is apparently induced by the infundibular region of the neural tube, as shown by the work of Hillemann (1943). The epithelial lobe, in turn, is necessary for the conversion of the saccus infundibuli into a pars nervosa. The epithelial lobe and the saccus infundibuli must maintain an intimate association to insure their normal development. Other parts of the neural tube, however, apparently have no influence on pituitary development, which proceeds normally in their absence. The evocating influence seems to be a chemical one, for even if the infundibular region is physically disorganized, its mere presence is sufficient to induce normal epithelial lobe development. Thus the chemical evocating influence is one which works by diffusion at close range. The organizing influence of the infundibulum seems to be present before the 33-hour stage in chick development. Stomodaeal ectoderm regenerated after the 33-hour stage is still capable of forming normal pituitary in the presence of the infundibulum. If the infundibular region of the 33-hour chick is destroyed, the posterior lobe of the pituitary fails to develop.



**Location of Primordial Tissue.** At the primitive streak stage of the chick embryo, the prospective hypophyseal ectoderm, mostly epithelial in origin, lies in the midline anterior to Hensen's node. Its position has been demonstrated by means of chorioallantoic grafts (Rudnick, 1932; Stein, 1933). Grafts taken from the same region during the head-process and early head fold stages are likewise capable of differentiating into hypophysis. However, the capacity for this tissue to differentiate is very low at early stages. In implants from donors of 8- to 12-somites, the hypophysis has a greater differentiating capacity, appearing in about 55 per cent of grafts; donors of 3 to 5 days' incubation yield grafts in 94 per cent of which hypophyseal tissue differentiates. At the 11-somite stage, the hypophyseal material is located in ectoderm closely attached to the ventral surface of the forebrain and just anterior to the oral plate ectoderm (Kingsbury, 1922; Stein, 1929).

#### *Morphogenesis of the Anterior Lobe*

The first sign of hypophyseal development is seen in the differentiated ectoderm which evaginates dorsalward from the roof of the oral cavity. This evagination is the beginning of Rathke's pouch or the hypophyseal pouch, which is first seen in the chick after 43 to 48 hours' incubation (Venzke, 1942). In the duck (*Anas platyrhynchos*), it has been observed at the stages of 15 somites (Adelmann, 1922), 19 somites, or 33 hours (Friedman, 1934), at 60 hours (Assenmacher, 1952), and after 3 days' incubation (Lups, 1929).

Friedman (1934) described the tissue as consisting of a plate of cells lying under the mesodermal mass anterior to the notochord. It is in intimate contact with the base of the forebrain anteriorly and with the oral membrane posteriorly. This position corresponds to the angle between the membrana buccopharyngea and the roof of the mouth (Lups, 1929) as shown in Fig. 335-A and B. The relation between the prechordal mesoderm and the hypophyseal ectoderm was traced by Friedman (1934), who suggested that Rathke's pouch is formed in the duck (*Anas platyrhynchos*) by the release of a mesodermal barrier over the ectoderm, accompanied by the rapid overgrowth of the forebrain. The pouch then expands laterally and in an upward direction but is retarded in the medial plane. The medial mesoderm, which is in close contact with the pouch, actually contributes to the hypophyseal anlage in the form of rods or knobs of cells which attach to the tip and sides of the pouch. With continued growth of the ectoderm, the mesodermal knobs disappear. Thus it becomes apparent that prechordal plate mesoderm does not directly enter into hypophysis formation since the mesodermal knobs are incidental accretions and soon disappear.

The development of Rathke's pouch continues by expanding upward,



and the mesoderm in its path migrates laterally. While the brain is in the process of overgrowth, the pouch approaches the anterior tip of the notochord in the 23- to 24-somite duck, *Anas platyrhynchos* (Friedman, 1934). As the upward expansion of the pouch continues, it becomes intimately connected with the mesoderm of the premandibular cavities. With the

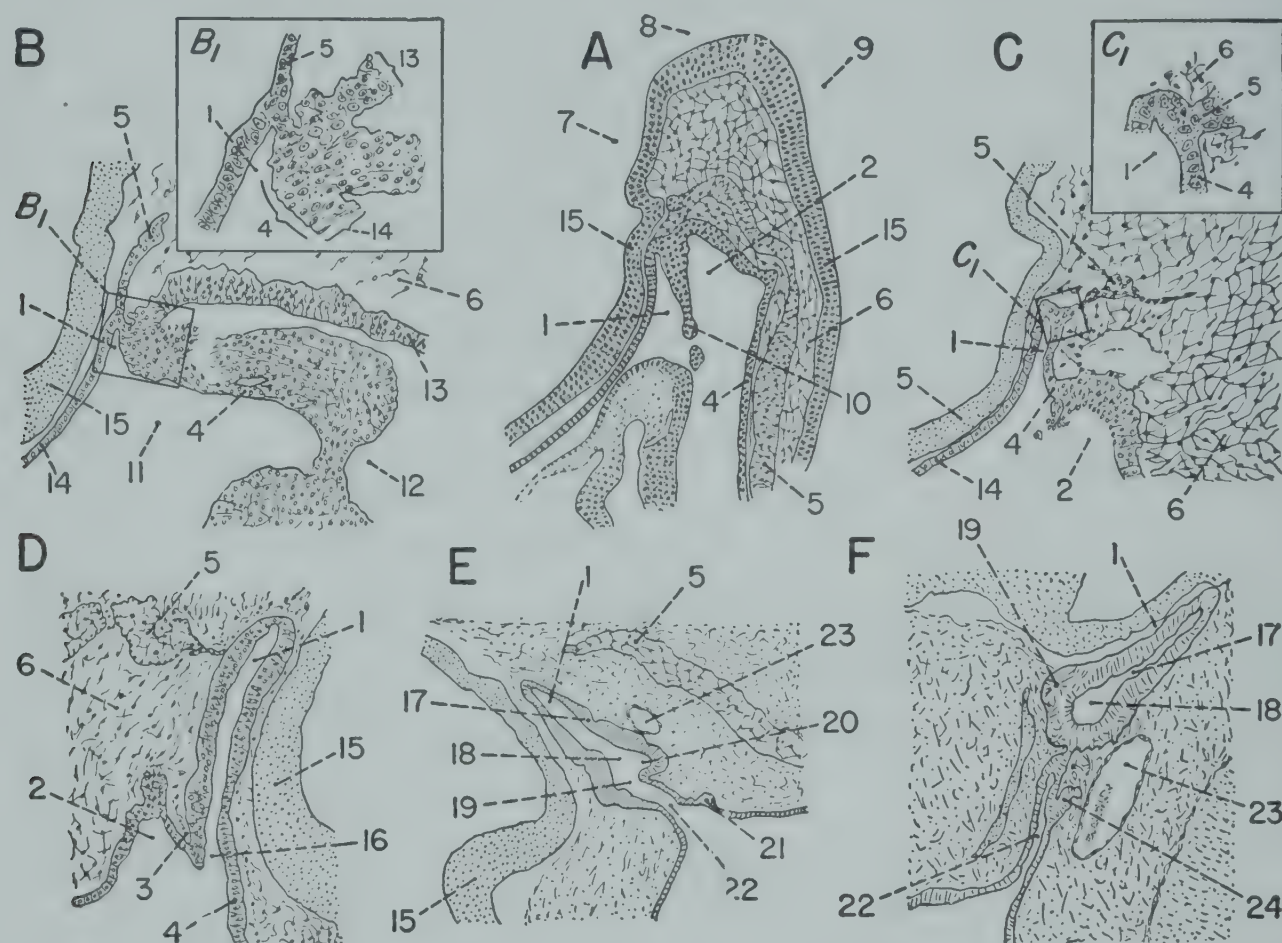


Fig. 335. Early morphogenesis of hypophysis in the duck, *Anas platyrhynchos*, and pigeon, *Columba livia*. (Redrawn with modifications A, after Economo, 1899; B to F, after Lups, 1929.)

A, median sagittal section through stomodaeal cavity of 2.5-day pigeon embryo ( $\times 50$ ); B, median sagittal section through stomodaeal cavity of 3-day duck embryo ( $\times 75$ ); *Insert B<sub>1</sub>*, detail of hypophyseal angle, or Rathke's pouch ( $\times 150$ ); C, median sagittal section through hypophyseal anlage of 3-day duck embryo ( $\times 75$ ); *Insert C<sub>1</sub>*, detail of Rathke's pouch ( $\times 150$ ); D, median sagittal section through hypophysis anlage of 5-day duck embryo ( $\times 50$ ); E, median sagittal section through hypophyseal pouch of 6-day duck embryo ( $\times 30$ ); F, median sagittal section through hypophyseal pouch of 7-day duck embryo ( $\times 30$ ).

1, Rathke's pouch; 2, Seessel's pocket; 3, epithelial mass; 4, buccopharyngeal membrane; 5, chorda dorsalis; 6, mesenchyme; 7, forebrain region; 8, midbrain region; 9, hindbrain region; 10, section through preoral gut; 11, mouth cavity; 12, gut cavity; 13, endoderm; 14, ectoderm; 15, wall of brain; 16, secondary opening of hypophyseal anlage; 17, constriction; 18, middle cavity; 19, anterior cavity; 20, ventral outpocketing; 21, remains of Seessel's pocket; 22, hypophyseal duct; 23, blood vessel; 24, dorsal process.

enlargement of the head cavity, the connection between that cavity and the pouch is established, and this connection has been interpreted as "proboscis pores." This is simply an interpretation of a close connection between the premandibular head cavities and Rathke's pouch, which cannot quite be considered a continuous lumen. There is never a common lumen between



the head cavities and Rathke's pouch. At the 30-somite stage, the mesodermal knobs mentioned earlier cluster at the tip of the pouch (Fig. 335-C).

The formation of lateral lobes at the lower end of Rathke's pouch indicates the beginning of the pars tuberalis. The lobes appear in the duck (*Anas platyrhynchos*) after 96 hours of incubation (Friedman, 1934). By this time, the pouch is divided by a small constriction into the main pouch and a lesser central portion. The lobes develop at the region of the constriction.

According to the classification of Woerdeman (1914), the hypophyseal diverticulum consists of an anterior and a middle chamber and the ventral pouch. The constriction separates Rathke's pouch from the more dorsal and anterior chambers. Pfeiffer (1925) classified the stomodaeal outpocketings of the 4-day chick embryo as consisting of Rathke's pouch, which is anterior and contributes to the hypophysis, and Seessel's pouch, which is the more posterior and does not contribute. The anterior pouch is divided into two parts which constitute the early hypophysis.

The glandular part of the hypophysis in the chick is formed from stomodaeal ectoderm, according to Pfeiffer (1925); whether or not endoderm enters into hypophysis formation is disputable. In the chick, Pfeiffer noted no contribution of endoderm. Lups (1929) observed an intimate connection between the two pouches in the duck (*Anas platyrhynchos*) and concluded that Seessel's pouch fuses in part with the hypophysis anlage. Friedman (1934), in a study of 75 duck embryos, found no such fusion, even though ectodermal and endodermal elements were in very close association.

A consideration of the early development of the hypophysis should include mention of the relationship of the chorda dorsalis to Rathke's pouch. Lups (1929) considered this a secondary association. There is some question as to whether the chorda contributes cells to the hypophysis anlage. In the chick (Atwell, 1915), the tip of the chorda retains its endodermal connections with Rathke's pouch, and Seessel's pocket is formed from the same endoderm. According to the observation of Atwell, the tip of the notochord and parts of the stomodaeum are endodermal. After the degeneration of Seessel's pocket, the chorda remains attached to Rathke's pouch.

In the 3-day chick embryo (Bruni, 1915), the pigeon, *Columba livia* (Economo, 1899), and the 4-day duck, *Anas platyrhynchos* (Lups, 1929) a thick fold of epithelium appears between Rathke's and Seessel's pouches (Fig. 335-D). This epithelial bridge is a continuation of the stomodaeal epithelium. At 5 days in the chick there is a common lumen (as a result of the absence of the epithelial partition) between Seessel's pocket and the hypophyseal diverticulum. Bruni (1915) noted an intermediate chamber



between the two pouches in the chick, but Lups (1929) was unable to find this chamber in the duck. The epithelial bridge projects into this so-called pharyngo-hypophyseal chamber.

*The pars tuberalis.* In a very short time, the pharyngo-hypophyseal chamber loses its identity, because Seessel's pocket becomes smaller until it is only a thickening of the epithelium. In the 6-day chick (*Anas platyrhynchos*) the chorda is no longer attached to the hypophyseal pouch by

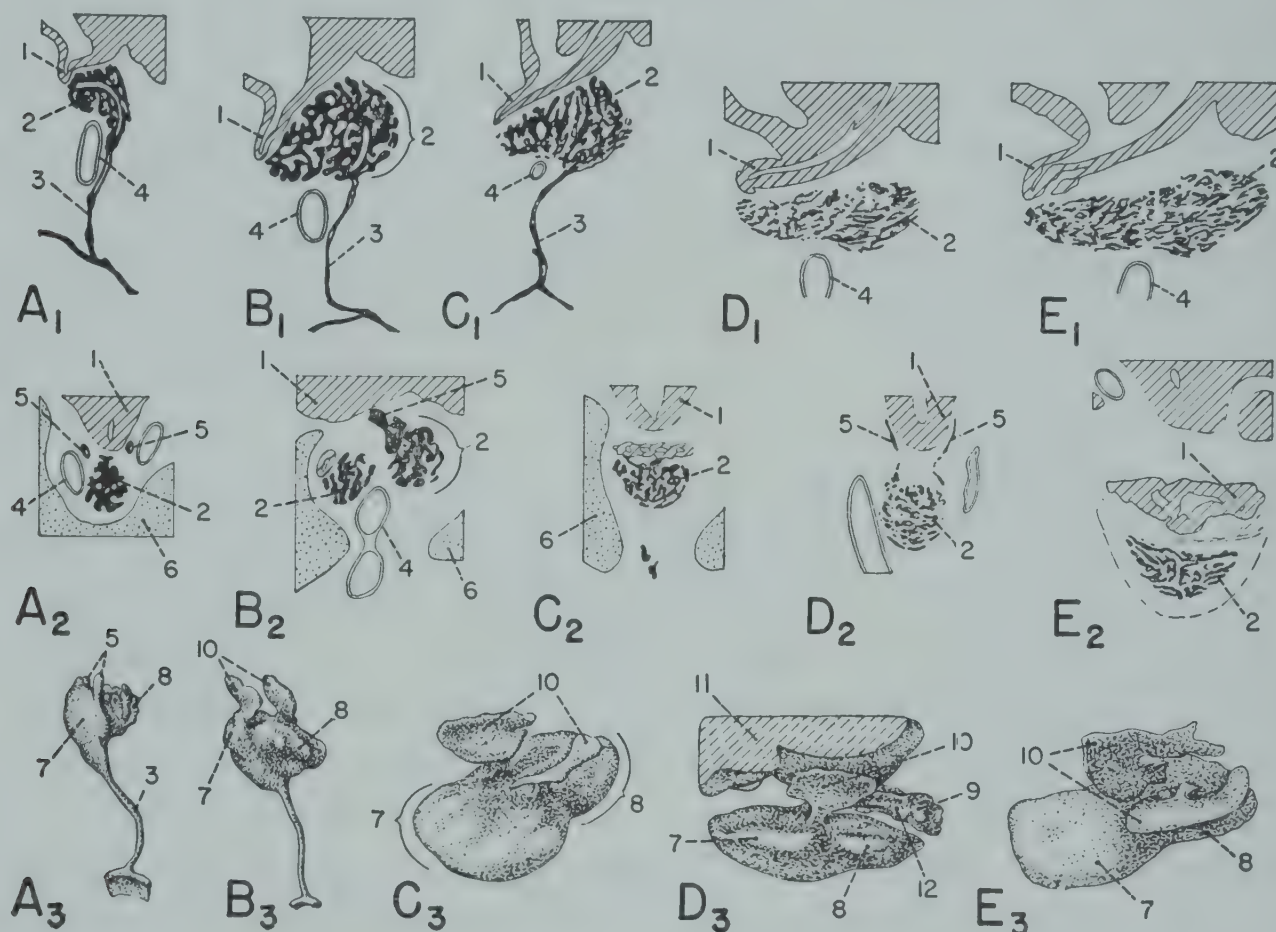


Fig. 336. Development of the hypophysis in the chicken embryo between 7 and 18 days' incubation. (Redrawn with modifications after Atwell, 1939.)

Row 1 across, diagrammatic representations of midsagittal sections of the hypophysis region; row 2 across, frontal sections of the above, except  $B_2$ , which is a paramedian sagittal section; row 3 across, wax plate reconstructions of the hypophysis at corresponding stages of incubation.

A series, from 7-day embryos; B series, from 10-day embryos; C series, from 13-day embryos; D series, from 15-day embryos; E series, from 17-day embryos.

1, brain wall and neural hypophysis; 2, epithelial hypophysis; 3, epithelial hypophyseal stalk; 4, internal carotid artery; 5, tuberal process; 6, sphenoid cartilage; 7, rostral end of hypophysis; 8, caudal portion of hypophysis; 9, neural lobe; 10, pars tuberalis; 11, optic chiasma; 12, infundibular process.

the mesenchymal strand, and Rathke's pouch has become constricted to form a central chamber and an anterior chamber (Lups, 1929) (Fig. 335-E). The same divisions in the chick have been called caudal and cephalic lobes (Rahn, 1939; Wilson, 1952), or caudal and rostral parts (Atwell, 1939). In the 6- and 7-day duck (*Anas platyrhynchos*) the two divisions are separated by a constriction (Fig. 335-E and F). From this constriction arise the paired lateral protuberances, the lobuli lateralis (Lups, 1929), or tuberal processes



(Venzke, 1942), which are the beginnings of the pars tuberalis (Fig. 336-A<sub>2</sub>, A<sub>3</sub>, and B<sub>2</sub>). In the chick, the tuberal processes have been said to appear after 60 hours of incubation (Venzke, 1942) and at 5 days 20 hours (Tilney, 1914). They are located just anterior and slightly medial to the internal carotid arteries. They are also juxtaneural in relation to the infundibular process and eminentia saccularis, which are both elements of the pars neuralis (Tilney, 1914).

In the 7-day chick (Atwell, 1939), the axes of the rostral and caudal parts of the hypophyseal anlage form a 90° angle. The caudal region is parallel to the floor of the brain, and the rostral part is parallel to the epithelial stalk (Fig. 336-A<sub>1</sub>). This stalk, which appears in the chick between the sixth and seventh days, connects the hypophysis to the roof of the mouth. After the disappearance of the stalk, the rostral part is oriented like the caudal part, and the gland then has a distinct slipper shape (Atwell, 1939) as shown in Fig. 336-D<sub>1</sub>. These parts are separated by the groove mentioned by Lups as a constriction.

The hypophysis gradually moves farther away from the roof of the mouth and reaches its definitive location at the base of the brain. Lups (1929) observed the distance between the pharynx and the anlage widening in the 7-day duck (*Anas platyrhynchos*). At the same time, the anastomosis of the two internal carotid arteries, which was caudal to the hypophysis, now becomes ventral to it.

The hypophyseal stalk appears in the 6-day duck (*Anas platyrhynchos*) as a small protuberance connecting the hypophysis to the roof of the mouth. This stalk is formed in the 4.5-day chick (Economo, 1899). Economo (1899) described the hypophyseal stalk as a solid cord of cells in the 7-day pigeon, *Columba livia* (Fig. 337-A). In a chick incubated 125 hours, Bruni (1915) noted two processes from the dorsal wall of the stalk. The upper process he called a median diverticulum, while the lower is the remnant of Seessel's pocket, according to Atwell and Sitler (1918). Lups (1929) observed one such process from the posterior wall of the hypophyseal stalk.

In the 9-day duck (*Anas platyrhynchos*), the hypophysis is more dorsal in position. Its dorsal surface is almost in contact with the tuber cinereum in the floor of the third ventricle of the brain. From the distal part of the hypophysis a dorsal outgrowth extends upward to join a tubular downgrowth from the floor of the brain. Together they form the pars nervosa. The contribution from the brain, namely, the process infundibuli (Fig. 337-B and C), appears in the 8-day pigeon (*Columba livia*) and the 10-day chick (Economo, 1899). By the chick's fourteenth day (Fig. 337-D), the lateral parts of the infundibular process are more glandular, and its stalk, which was previously entirely epithelial, is surrounded by nerve fiber tissue (Economo, 1899).



By the chick's sixth day, the caudal region of the pars distalis is well established. The dorsal part of the caudal region is maintained as the thickened dorsal wall of the original Rathke's pouch.

By the eighth day, conspicuous cords appear on the dorsal and lateral surfaces of the anterior part of the pars distalis, and soon they also appear anteriorly and ventrally. The cell cords represent an incipient cephalic region and they extend anteriorly to the dorsal attachment of the tuberal processes and to the ventral attachment of the epithelial stalk (Wilson, 1952). Connective tissue increases, especially in the cephalic region.

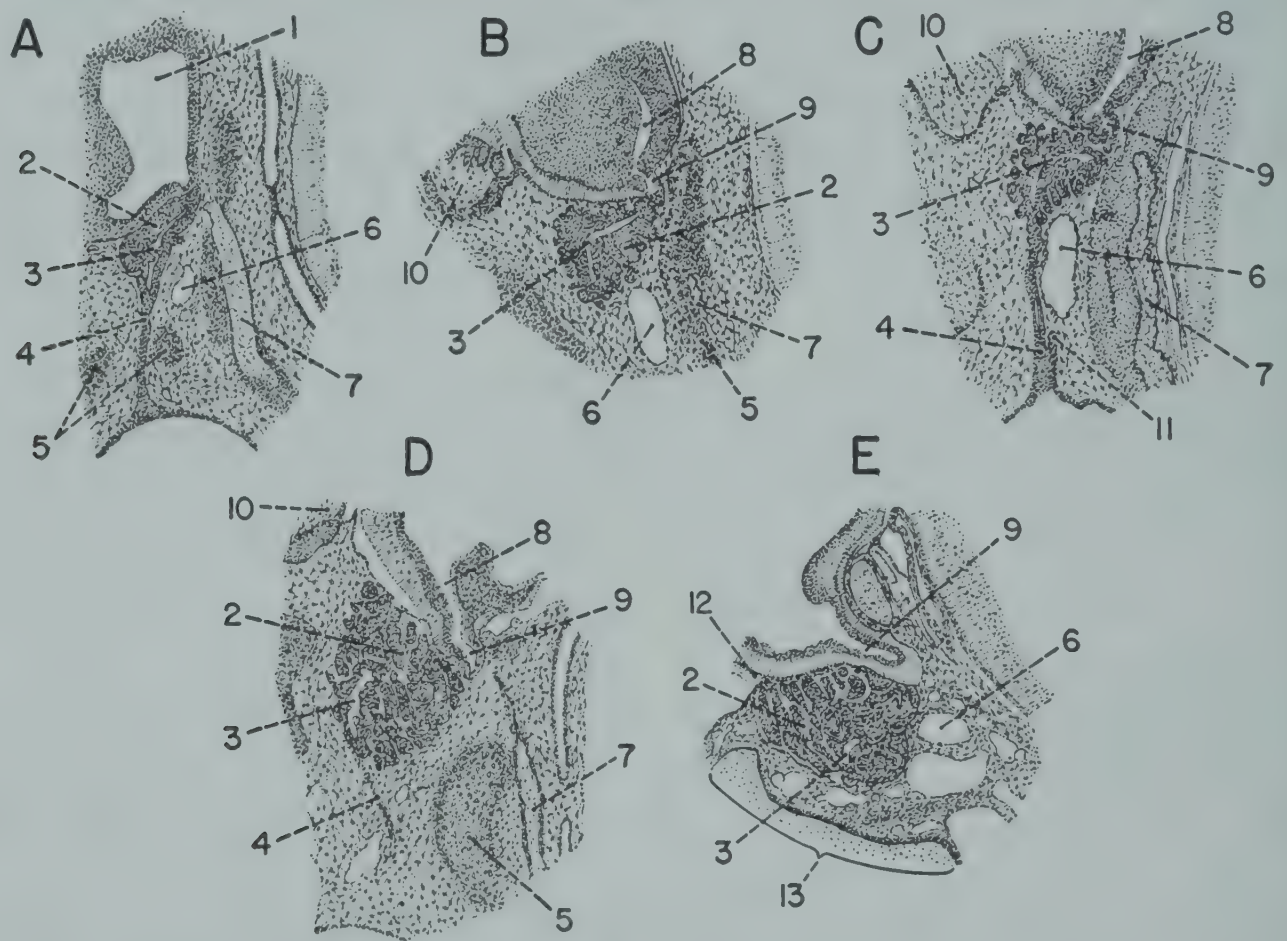


Fig. 337. Further development of the avian hypophysis as seen in the pigeon, *Columba livia*, and the chicken, *Gallus gallus*. (Redrawn with modifications after Economo, 1899.)

A, 7-day pigeon embryo; B, 8-day pigeon embryo; C, 10-day chicken embryo; D, 14-day chicken embryo with hypophysis definitely tubular; E, pigeon at hatching, hypophysis is in intimate contact with brain floor. All  $\times 25$ .

1, midbrain; 2, hypophysis; 3, lumen of hypophysis; 4, hypophyseal duct; 5, sphenoid cartilage; 6, carotid; 7, chorda dorsalis; 8, infundibulum; 9, infundibular process; 10, optic chiasma; 11, remains of Rathke's pouch; 12, pia mater; 13, sella turcica.

The hypophyseal epithelium within the gland is arranged perpendicular to the floor of the brain and residual lumen in the 13-day duck (*Anas platyrhynchos*) embryo (Painter, 1942). Interspersed between the epithelium are mesenchymatous strands and blood vessels. In the next few days the gland becomes more compact, the caudal part is pointed, and the neural and epithelial lobes are separated by connective tissue. This is similar to the picture after hatching, although in the duck, the pars tuberalis may be a discontinuous island of cells (Painter, 1942).



A connective tissue sheath, which began forming around the pars distalis in the 13-day chick embryo, is a dense capsule around the gland by the sixteenth day.

The anlage of the hypophysis at first has a well-defined lumen which gradually becomes discontinuous and is finally obliterated. It is continuous in all 6-day chick glands and is discontinuous on the following day (Wilson, 1952). Atwell (1939) observed complete lumina until 7 days, discontinuous spaces at 9 days, and a complete absence of lumen after 11 days. In the

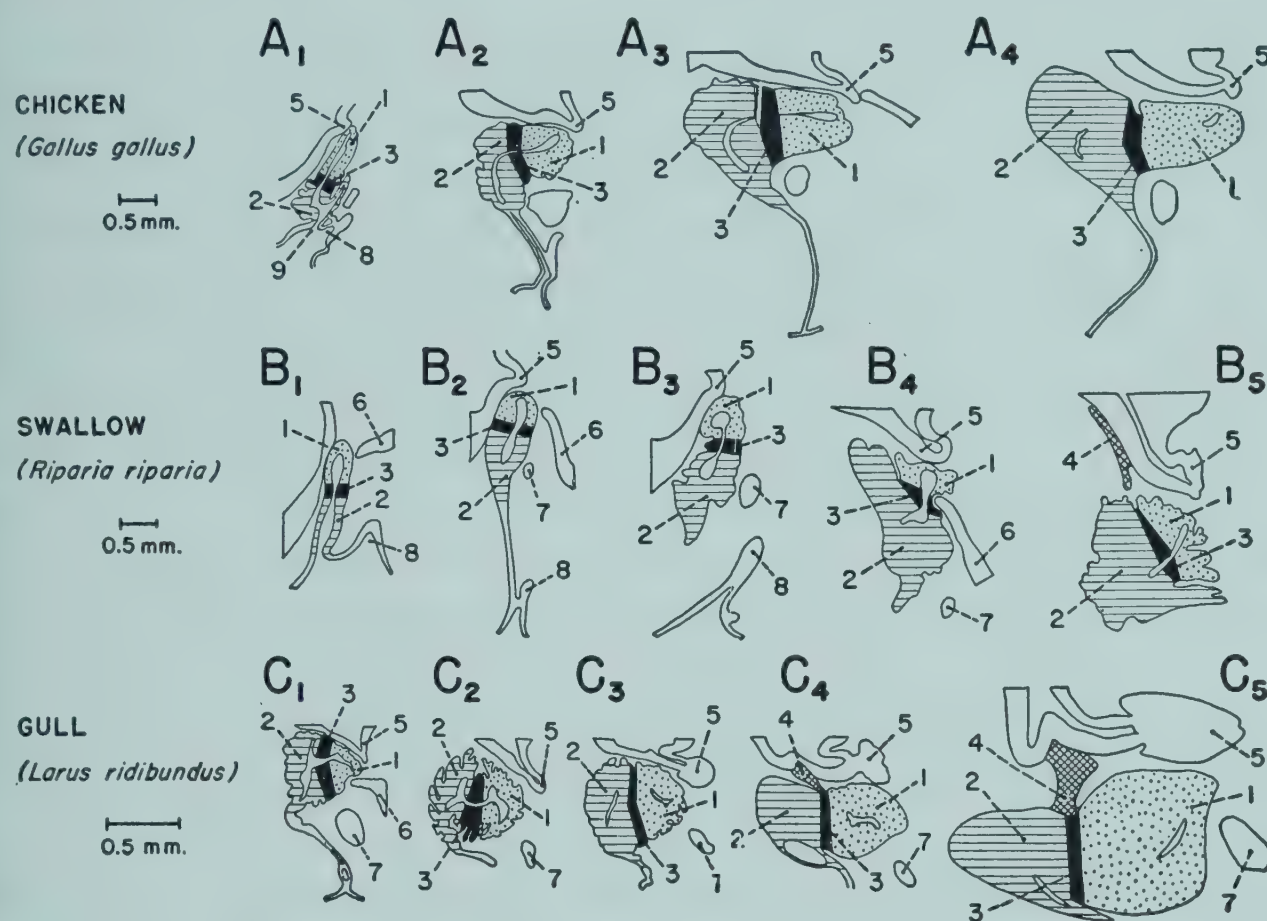


Fig. 338. Comparative study of the morphogenesis of the adenohypophysis of three species of birds. (Redrawn with modifications after Wingstrand, 1951.)

A series, chicken embryo (*Gallus gallus*): A<sub>1</sub>, 5 days; A<sub>2</sub>, 7 days; A<sub>3</sub>, 11 days; A<sub>4</sub>, 13 days; B series, swallow embryo (*Riparia riparia*): B<sub>1</sub>, 6 mm. (crown-rump length); B<sub>2</sub>, 7.8 mm.; B<sub>3</sub>, 8.5 mm.; B<sub>4</sub>, 10.5 mm.; B<sub>5</sub>, 19 mm. (hatching stage); C series, gull embryo (*Larus ridibundus*): C<sub>1</sub>, 20 mm. (crown-rump length); C<sub>2</sub>, 28 mm. (crown-rump length); C<sub>3</sub>, 60 mm. (total length); C<sub>4</sub>, 78 mm. (total length); C<sub>5</sub> young bird, 28 mm. All in scale.

1, aboral lobe; 2, oral lobe; 3, constricted area between lobes; 4, pars tuberalis; 5, saccus infundibuli; 6, notochord; 7, intercarotic anastomosis; 8, Seessel's pocket; 9, Rathke's pouch.

9-day duck (*Anas platyrhynchos*), the residual lumen extends to the epithelial stalk. At this time, the stalk is solid and still reaches to the roof of the mouth (Painter, 1942). At 10 days, the residual lumen extends the full length of the hypophysis, and the gland is in a more horizontal position than in the 7-day chick. Painter (1942) pointed out that the duck hypophysis never assumes the right angled or L-shaped configuration, nor does the swallow, *Riparia riparia*, or the gull, *Larus ridibundus* (Wingstrand, 1951), as shown in Fig. 338.



The hypophyseal stalk, which reaches the roof of the 9-day duck (*Anas platyrhynchos*) embryo's mouth, is massive at the 10-day stage (Painter, 1942). In the 17-day duck (Painter, 1942) and the 9-day chick (Rahn, 1939), the stalk is much shorter, its lumen is discontinuous, and degeneration has begun. At 23 days, the residual lumen disappears in the duck, and the stalk itself atrophies on the following days. In the pigeon (*Columba livia*) embryo, the lumen has disappeared by the eighth day (Economo, 1899), and the connective tissue capsule is continuous around the gland.

In the development of the pars tuberalis, each tuberal bud, at one stage, has a lumen which is not connected with the rest of the hypophyseal tract. The lumina are present in the 7-day chick embryo (Atwell, 1939), but they have disappeared by the eleventh day. By this time, the tuberalis tissue is in flat plates spread out under the brain floor (cf. Fig. 336-D<sub>3</sub>). By the sixteenth day (Tilney, 1914), the tuberal processes have fused across the median line, thus displacing the pars distalis from its original juxtaneural position. The newly fused body is the definitive pars tuberalis, and it occupies the position in which it is found in the adult bird.

The neural lobe or infundibular process is well formed by the seventh day, and at that time it has a large cavity continuous with the third ventricle of the brain. The distal part of the lobe is wide and attached to the infundibulum of the brain by a narrow stalk (Atwell, 1939). In the 16-day chick embryo, the pars nervosa is partly connected with the pars distalis by a reduced residual lumen (Tilney, 1914).

Subsequent to the time of hatching, the hypophysis in the pigeon (*Columba livia*) is ovoid with the pointed end directed anteriorly. The upper surface of the gland is in contact with the floor of the midbrain. The only part of the hypophyseal cavity remains in the anterior end of the hypophysis (cf. Fig. 337-E). In the lateral expansions there is a lumen. The entire body is surrounded by a connective tissue capsule. Connective tissue grows in below the floor of the brain and presses down the hypophysis; in doing so, it squeezes the two later hypophyseal masses out of existence and leaves the hypophysis in the adult pigeon (*Columba livia*) suspended by a stout stalk of connective tissue. This stalk is highly vascularized by blood vessels from the pia mater. Between the vessels, the hypophyseal strands extend up to the brain. The vessels fan out in the hypophysis and surround the hypophyseal strands. The hypophyseal cavity disappears entirely and the strands are solid. In the dorsal posterior part of the hypophysis, the cells are so closely packed as to appear as heaps of polygonal cells rather than strands. In the deeper part of the hypophysis the polygonal cells line up in strands which form a network. The entire gland, and especially the deeper portion, is well vascularized (Economo, 1899).



### *Innervation of the Hypophysis*

The principal nerve fibers passing from the brain to the pituitary are those of the hypothalamico-hypophyseal fasciculus. The nerves of this tract arise in a region dorsal to the optic chiasma and from a nucleus in the lower wall of the third ventricle (cf. Fig. 334-A<sub>2</sub> and C). The major portion of the innervation of the hypophysis is in the infundibular process or pars nervosa. The study by Drager (1945) showed that some nerve fibers terminated in the pars tuberalis, but apparently none innervate the pars distalis. Arteries adjacent to the hypophysis are accompanied by nerve bundles which often possess small ganglionic masses along their course; however, even these do not enter the pars distalis.

### *Cytology of the Hypophysis*

At first the cells of the hypophyseal anlage are relatively unspecialized epithelial cells. In the 9- to 12-day duck embryo (*Anas platyrhynchos*), the only distinct cell type is the stratified columnar epithelium which lines the residual lumen (Painter, 1942).

After differentiation has occurred, there are three major cell types in the pituitary gland. The chromophobes are the precursors of the acidophiles and the basophiles. The exact time of differentiation of any of these cells is difficult to settle, as much depends on histological techniques.

Basophiles usually appear before acidophiles. Wilson, M. E. (1948) noted basophilic cells in the anterior lobe of the chick's hypophysis on the sixth day of incubation. Moscona and Moscona (1952) observed a few chromophilic cells in the anterior lobe on the tenth day of incubation.

The first visible basophilia in the chick has also been reported to occur at 8 days (Rahn, 1939; Dawson, 1947) throughout both lobes. It is said that cells are undifferentiated in the duck (*Anas platyrhynchos*) at 9 to 12 days, and that acidophiles and basophiles both appear at 16 days (Painter, 1942).

In the duck (*Anas platyrhynchos*), both cell types are similar in size and possess eccentric nuclei. They also have distinct granules at the distal ends of the cells, and the cells are approximately equal in numbers. They differ, of course, in their characteristic staining reactions. On the seventeenth day, basophiles become less numerous, and they are almost absent on the nineteenth day. At the same time, acidophiles increase in number and become evenly distributed throughout the pars anterior. Within a few days after hatching they move to the caudal lobe. At this time basophiles are again visible in the cephalic lobe and, as before, are similar to the acidophiles in size and shape. Basophiles subsequently increase in size, number, and distribution.

The picture in the chicken embryo is somewhat different. The first



basophiles, seen at 6 days, have blackened granules and cell processes, and are immature cells. After 8 days, the larger, rounder basophiles appear in the cephalic lobe. During the last week of incubation, the basophiles of the caudal lobe begin their final differentiation. However, some of the immature caudal basophiles acquire an acidophilia after the eighteenth day and transform into caudal acidophiles (Wilson, M. E., 1949).

In the pigeon, *Columba livia* (Schooley and Riddle, 1938), granules appear in acidophiles between the twelfth and the sixteenth day of incubation. By the latter time, the cells are still vesicular and have clear, structureless cytoplasm. This is their condition until the time of hatching.

Whenever basophiles appear first and acidophiles follow, the former are probably transitory types of chromophobes, while the latter are clearly definitive cells. In fact, the acidophiles arise from the "basophilic chromophobes," according to Rahn (1939). The formation of acidophiles is described as occurring in a wave which spreads posteriorly from the most anterior region of the cephalic lobe. By the eighteenth day, the entire embryonic chick pituitary gland is filled with deep-staining acidophiles, but soon they become restricted to the caudal lobe. Beginning on the sixteenth day, pycnosis is common in the nuclei of acidophiles. Payne (1946), studying the chick, found that the pituitary is not predominantly acidophilic until 3 days after hatching. Furthermore, he distinguished two different kinds of acidophiles which differ in staining reactions and location within the pituitary.

Basophiles are scarce between the twelfth and eighteenth days of incubation, and fully differentiated cells of this type were not observed by Rahn (1939) until several weeks after hatching, although Payne (1946) noted them 10 days after hatching. Similarly, in the pigeon (*Columbia livia*), fully differentiated basophiles are not formed until sexual maturity is reached (Schooley and Riddle, 1938).

### *Hormonal Activity*

On the chick's fifth incubation day, there is physiological evidence for the secretion of intermedin, even though there are no recognizable associated cytological changes in the pituitary (Chen, Oldham, and Geiling, 1940; Kleinholz and Rahn, 1940; Rahn and Drager, 1941). Intermedin, so named because it is produced by the pars intermedia, is formed in the pars anterior in birds and other vertebrates that lack the pars intermedia. Intermedin is also known as the melanophore-dispersing hormone, so named because this is one of its functions.

According to Fugo (1940), whose conclusions are based on the results of hypophysectomy, the embryonic pituitary has no effect on primary morphological differentiation. The gland becomes active during the second half of incubation, secreting hormones which seem to exercise growth-promoting



thyrotropic and gonadotropic functions during embryonic life. The pars anterior, after hatching, contains pressor, oxytoxic, and antidiuretic components, while the melanophore principle is concentrated in the pars anterior (*DeLawder, Tarr, and Geiling, 1934*).

The pituitary gland has been said to exert no significant effect on the embryonic development of the other endocrine glands (*Ancel, 1937; Fugo and Witschi, 1938*). Ancel (1937) found that hypophysectomy of chick embryos by X-ray did not alter the normal development of adrenals, thyroid, parathyroids, and gonads. These are all controlled by the hypophysis in postnatal life. Fugo and Witschi (1938) also observed only a general delay in growth following hypophysectomy of 36-hour chicken embryos; the endocrine system was not unduly affected.

Evidence has also been presented to show that the embryonic pituitary exerts a definite effect on other endocrine organs. By transplanting anterior pituitary lobe on chick chorioallantois on the eighth day of incubation, Studitskii (1938) obtained an accelerated morphogenesis of the host's thyroid gland. He even noted a response of the chick embryo thyroid gland to transplanted pituitary on the eighth day of incubation (*Studitskii, 1941*). The transplant further produced hypertrophy, vacuolization of protoplasm, and lipoid infiltration in the parathyroids. In the pigeon (*Columba livia*)—which, according to Benazzi (1929), hatches with a nonfunctioning pituitary—a cytological examination reveals signs of function in the hypophysis and other endocrine glands on the eleventh day of incubation.

The interrelation between pituitary and thyroid activity has been established as beginning in the 11-day chick embryo. Martindale (1941) drew this conclusion from results of hypophysectomy and grafting experiments in which the thyroid actively produced colloid at 11 days in normal chick embryos, but not until later in hypophysectomized embryos. In the latter, growth and differentiation of the parathyroids were delayed as well. Furthermore, he found that the transplantation of a nondifferentiated thyroid gland from a hypophysectomized 12-day embryo to a normal 8-day embryo may cause the thyroid to start normal development on the eleventh day. There is a marked effect on the host's thyroid when hypophysis tissue is transplanted, if the host is an embryo of 10 days or more. There is some reaction even in 8- and 9-day embryos. The parathyroids as well as the thyroid react to the transplanted hypophysis by an increase in size and vacuolization of protoplasm (*Studitskii, 1946*).

## THE PANCREAS

The pancreas is an organ of dual function. In addition to its exocrine role as a digestive organ, it contains endocrine tissue in the form of islets



which secrete the hormone, insulin. This hormone is an important regulator of carbohydrate metabolism.

The morphogenesis of the pancreas is discussed in Chapter 6, which is concerned with the organs of the digestive tract, since the pancreas is an appendage of the fore-gut. Potvin and Aron (1927) noted the initial differentiation of the islands of Langerhans in the 8- and 9-day chick pancreas. The islets probably begin active secretion of hormone around the eleventh day of incubation (*Lillie, 1919*).

### THE PINEAL GLAND

The pineal gland, or epiphysis, is included here simply because it is sometimes thought of as an endocrine gland. However, it has never been shown to have any secretory significance, and no one has even suggested a plausible function of this apparently vestigial organ. It is perhaps best known as representing a structure homologous to the parapineal organ or third eye of certain primitive vertebrates.

The epiphysis forms as a dorsal protuberance of the diencephalon. It is formed in the 3- to 4-day chick embryo and arises from a region dorsal and posterior to the velum transversum (cf. Fig. 93, Chapter 4). Soon the bud elongates and the epiphysis then appears as a long, narrow tube lying dorsal to the diencephalon (cf. Fig. 97, Chapter 4). The tube is covered by many small hollow buds and the distal end is dilated.

### THE GONADS

The embryonic development of the ovaries and testes has been traced in Chapter 10. During the early stages, the male and female gonads are morphologically indistinguishable, but with sexual differentiation, there is evidence of hormone production.

As has already been mentioned in the discussion of the pituitary gland, the primary morphogenesis of the gonads seems to proceed without the assistance of hormones. However, sex hormones are released by the embryonic gonads quite early, as shown by the development of secondary sexual structures (cf. Chapter 10). For example, the androgens of the male are necessary to bring about regression of the oviduct which normally forms in male embryos (*Willier, 1952*). In both sexes, hormones seem to influence appropriate development of the genital tubercle, syrinx, and other secondary characteristics.

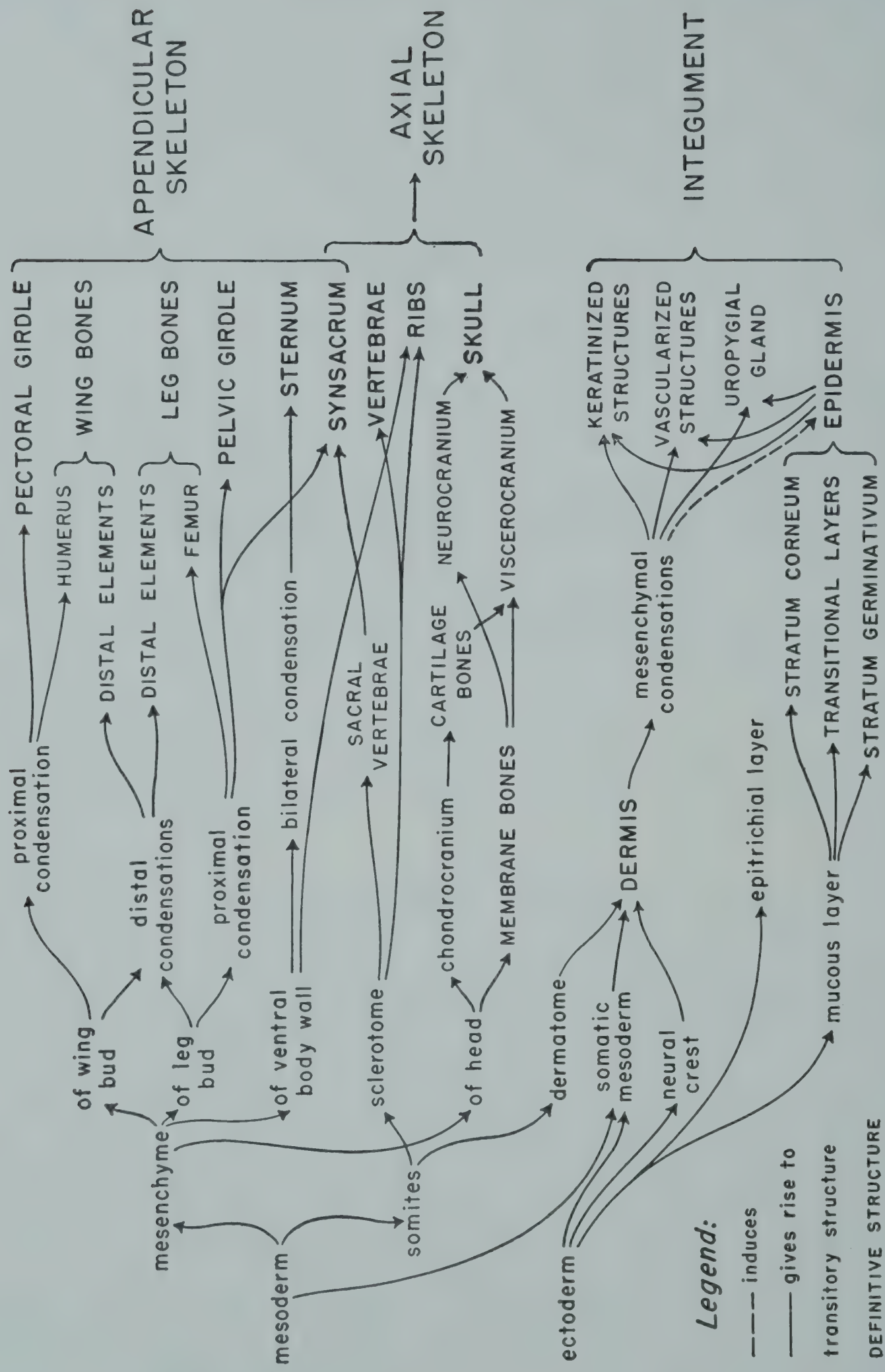


## CHAPTER TWELVE

# *The Skeletal System and the Integument*

*On bones depends the body's standing,  
The nature of the creature's form,  
The higher vertebrates commanding . . .  
Both skin and feathers keep birds warm.*







# THE SKELETAL SYSTEM AND THE INTEGUMENT

The skeleton is the supporting framework which serves as a protection for the soft tissues and internal organs of the embryo. The integument, including the skin and its appendages, also functions in a protective capacity and is involved in the adjustment of the organism to external conditions. Organogenesis of both the skeletal system and the integument is a process that continues after hatching. Certain bones do not ossify until post-hatching; likewise, portions of the integument, such as the down feathers, are replaced by related structures after hatching.

## THE SKELETAL SYSTEM

The supporting structure of the avian embryo is comprised of the cartilaginous skeleton derived from aggregations of mesenchyme from which the bony skeleton is formed by ossification. The formation of cartilage or chondrification does not take place simultaneously in all parts of the embryo since condensations arise at various times. In a single bone primordium the chondrification process may be in several different stages of completion. However, before ossification begins, generally the entire structure is cartilaginous.

Besides the cartilaginous bones, which are the most numerous, there are membrane bones, such as some of the cranial bones, the clavicle, and the uncinat processes of the ribs, which originate directly in membrane and ossify without going through the cartilaginous stage.

The ossified skeleton consists of the axial portion, or the head and trunk bones, and the appendicular skeleton, or limb supports, with the pelvic and pectoral girdles uniting this structure to the axial skeleton.

### Osteogenesis

The first step in the formation of the bones of the skeleton, whether membrane or cartilage bones, is a condensation of mesenchyme. In the development of a membranous bone, mesenchymal condensation is followed directly by bone deposition. The most important membranous bones are those comprising the vault of the skull. In the development of a cartilaginous bone, cartilage differentiates in the condensed mesenchyme



and is then eroded and replaced by osseous and marrow tissue; in effect, the cartilage acts as a model or mold for the bone. Phylogenetically, of course, the cartilaginous bones are older than the membranous bones, and they are much more numerous. Their ossification is both perichondral (beneath the perichondrium, or external investing membrane of fibrous connective tissue) and endochondral, that is, within the substance of the cartilage. In birds, perichondral ossification predominates, except in the vertebrae.

### *Membrane Bones*

Studies of the development of membrane bones in birds have been concerned in the main with cranial bones, especially the frontal bone, and with the elements that make up the largest part of the mandible (the angulare, supra-angulare, operculare, and dentale).

In membrane bones, ossification occurs directly in areas of condensed mesenchyme which fix radioactive sulfur to a greater extent than the neighboring regions (*Amprino, 1955a*). At first, all the cells composing such an area are very similar to those of the surrounding tissue; then the cells in the center of the mass differentiate into osteoblasts, a change that is indicated by their enlargement and an increase in the affinity of their cytoplasm for stains (*Fell and Robison, 1930*). Under the electron microscope, some of the cells are seen to contain long filaments, but most of them are filled with short filaments, granules, and mitochondria (*Jackson, 1954*).

Very shortly, delicate osteogenic fibers appear among the osteoblasts (*Fell and Robison, 1930; Amprino, 1955a*); in the mandible, these are first seen in the region near the articulation of the jaw, on the sixth day of incubation, and their differentiation progresses toward the apex, where osteoid tissue is present by the ninth day. As the fibers increase in number, they form irregular lamellae (*Fell and Robison, 1930*). The electron microscope shows that the lamellae consist of closely packed fibrous material. At first, the area of bone matrix is indistinguishable from the cytoplasm of the cells, but it gradually becomes composed of long, parallel fibrils connected together by finer filaments (*Jackson, 1954*). When cultures derived from the frontal bone of the chick embryo are viewed with polarized light or phase-contrast illumination, the fibers appear to be entirely extracellular and to have no connection with the cytoplasm of the cells (*Rodova, 1948*).

As blood vessels grow into the ossification center, the osteoid tissue acquires a trabecular structure (*Fell and Robison, 1930*). It is probable that the trabecular architecture is determined by the blood vessels, for, in cultures of the chick embryo's frontal bone grown on the chorioallantoic membrane, there is a close relationship between the vascular and trabecular



patterns (*Hancox, 1948, 1949b*). It is also interesting to note that the healing of defects in fragments of frontal bone cultivated in this manner is not dependent upon the presence of a nerve supply (*Hancox, 1949b*). The trabeculae of membrane bone are highly radioactive in embryos treated with  $S^{35}$  (*Amprino, 1955a*).

The bone matrix increases in amount, density, and homogeneity, and many osteoblasts become enclosed in it and are transformed into osteocytes.

Meantime, on the chick's eighth day of incubation, small areas of calcification appear near the articular end of the jaw; calcification has extended to the apex of the jaw by the tenth day, and the entire mandible is heavily calcified by the twelfth day (*Fell and Robison, 1934*). The radioactivity of membrane bone into which  $S^{35}$  has been incorporated decreases somewhat after decalcification. A part of the isotope taken up by the bone seems to become fixed to the mineral components through ionic exchange (*Amprino, 1955a*).

Simultaneously with the increase in the amount of bone matrix, bone is resorbed and redeposited in the mandible. As a result, a large marrow cavity is excavated throughout the length of the jaw by the chick's fifteenth day of incubation (*Fell and Robison, 1930*).

In both membrane and cartilage bone, phosphatase activity parallels ossification. There is a small amount of phosphatase activity in the chick embryo's mandible on the sixth and seventh days of incubation, when osteogenic fibers are first seen; and there is a constant increase thereafter. The ratio of phosphatase to bone weight also increases and has attained a value on the fifteenth day that is one and one-half to two times as large as the maximum value for cartilage bone (*Fell and Robison, 1930*).

### *Cartilage Bones*

The cartilage of which embryonic bone rudiments are composed is of the hyaline type. It consists of a translucent matrix which ordinarily appears homogeneous but which is shown by certain methods to be largely made up of fibrils and fibers of collagen. The matrix is basophilic, largely because of the presence of abundant chondiomucoid. At rather widely spaced intervals, cartilage cells are embedded in the matrix, which often forms a particularly dense and slightly striated capsule immediately around each cell; capsules, however, are now well differentiated in avian embryos. Cartilage is characteristically surrounded by a fibrous membrane, the perichondrium, in which flattened cells are interspersed among fibers that run parallel with the surface of the cartilage. Peripherally, the perichondrium merges with the surrounding connective tissue.

**The Formation of Cartilage.** In the formation of embryonic cartilage, several stages can be recognized (Fig. 339). First, a condensation of un-oriented mesenchymal cells appears in the region of the future bone; then



the nuclei in the condensed area become aligned so that their long axes are parallel. Tissue in either or both of these stages is termed precartilaginous or is referred to as a mesenchymal, precartilaginous or prechondral blastema. Almost simultaneously with the orientation of the nuclei, the first traces of matrix appear between the cells. From this time until the matrix completely encircles every cell, the tissue may be considered as being in a prochondral or protochondral stage, although it is frequently classed as still precartilaginous or prechondral. Next, the cells enlarge and become round, and the

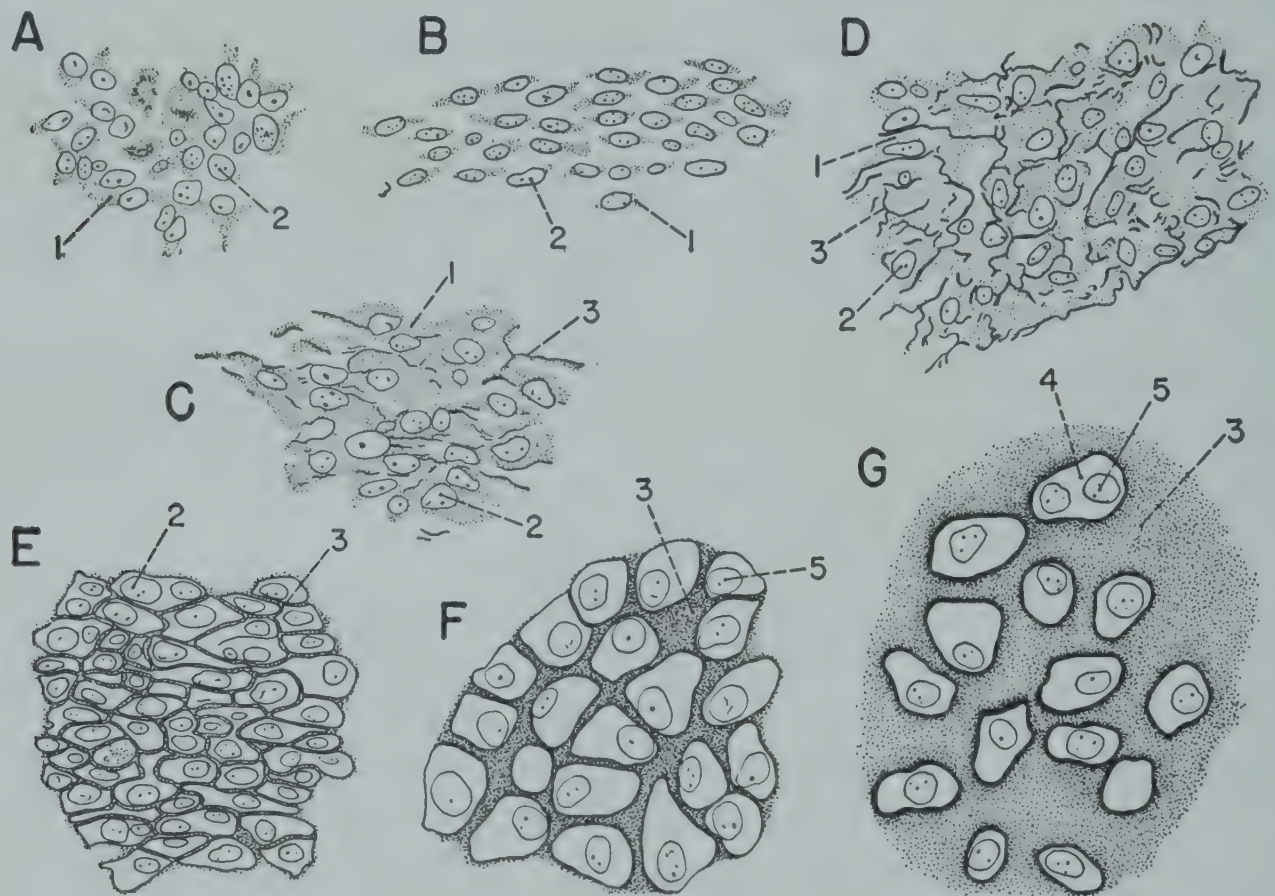


Fig. 339. Stages in chondrogenesis, as seen in the duck (*Anas platyrhynchos*) embryo from 6 to 11.5 days' incubation. (Redrawn with modifications after Blinov, 1938.)

A, mesenchymal condensation without orientation of nuclei; B, mesenchymal condensation with elongation and parallel alignment of nuclei; C, D, E, prechondral stages, with appearance of intercellular matrix in increasing amounts until it completely encircles the cells; F, metachondral or hypertrophic stage, with enlargement of cells and formation of additional matrix; G, embryonic cartilage. All  $\times 500$ .

1, chondroblast; 2, chondroblast nucleus; 3, matrix; 4, lacunae; 5, chondrocyte nucleus.

amount of matrix greatly increases; this phase is generally known as that of hypertrophic cartilage. The last stage, that of fully differentiated embryonic cartilage, is characterized by a still larger amount of matrix.

It has been shown by the use of stains (Weel, 1948) and of radioactive sulfur,  $S^{35}$  (Amprino, 1955a), as sulfate, that the chemical differentiation of the cells that are destined to form skeletal elements precedes their morphological differentiation. In chick embryos treated with the sulfur isotope early in incubation, the radioactivity of the axial part of a limb primordium, for example, is considerably greater than that of the surround-



ing regions at the time when the cartilage is still in first or blastematos stage. The high concentration of sulfur in the skeletogenous tissue probably indicates that chondroitin-sulfuric acid (a constituent of chondromucoid) is already being synthesized. The uptake of radioactive sulfur parallels the degree of differentiation of the cartilage. Prochondral anlagen, such as those of the vertebrae and cranial bones, incorporate more of the isotope than their respective prechondral blastemata, and the quantity of radioactive sulfate fixed per unit volume of tissue rises with the increase in the basophilia and amount of the matrix. A diminution in radioactivity accompanies the hypertrophy of the cartilage cells, because their enlargement immediately reduces the amount of intercellular substance per unit of volume of tissue (*Amprino, 1955a, 1955b, 1955c*). From now on, there is a decline in the rate of  $S^{35}$  fixation, as shown by the accompanying data from

Age of Chick Embryo (days)	Amount of $SO_4$ Ion Fixed in Center of Tibial Shaft (micrograms per 100 mg. wet tissue)
8	32
10	22
11	11.7
12	7.7
13	1.9
16	1.2
18	1.0
21	0.65

experiments on cartilage *in vitro* (*Layton, 1950*). In regions where perichondral ossification occurs, the continuing decrease in the amount of sulfur taken up by cartilage probably indicates that the hypertrophied chondrocytes gradually lose the ability to metabolize chondroitin-sulfate, although the sulfur fixed prior to cellular enlargement is retained until the cartilage breaks down. In areas (such as the anlagen of vertebrae and many cranial bones) where ossification is endochondral, the sulfate fixed in early stages is removed before the cartilage is destroyed, but the capacity to incorporate new sulfate is maintained to some extent (*Amprino, 1955a*). The uptake of  $S^{35}$  by chick embryonic cartilage (condyles of long bones) is inhibited *in vitro* by anaerobic conditions and by the presence of substances that suppress respiration. The synthesis of chondroitin-sulfate, therefore, is apparently dependent on respiration, which is about 200 times as high in embryonic as in adult cartilage; unlike the latter, embryonic cartilage contains cytochrome oxidase. Glucose markedly stimulates sulfate fixation without increasing respiration, probably because it accelerates the synthesis of the carbohydrate precursor of chondroitin-sulfate. The usual enzymes of carbohydrate metabolism appear to be present in embryonic cartilage (*Boyd and Neuman, 1954*).



Chondrogenesis does not begin simultaneously nor proceed at the same rate in all regions; in general, the earlier histogenesis starts, the more slowly it takes place, with the result that cartilage formation is completed at approximately the same time in most of the bone primordia. The accompanying tabulation (*Blinov, 1937, 1938*) compares the time required for chondrogenesis in a number of elements of the duck (*Anas platyrhynchos*) embryo's cartilaginous skeleton.

The study of the histogenesis of cartilage bones in birds has been limited chiefly to observations on the long bones of the chick embryo. The condensation of mesenchyme begins in the proximal part of a limb primordium, and a compact mass of cells is soon formed (*Johnson, 1883; Fell, 1925*). In the cells of this mass, the nuclei are round, contain two nucleoli, and are surrounded by a small amount of cytoplasm, which is drawn out into fine radiating processes; the cytoplasmic processes of adjoining cells are in continuity (*Fell, 1925; Jackson, 1954*). The Golgi apparatus is reticular, and the mitochondria differ from those of the mesenchyme cells in that they are rodlike rather than filamentous (*Weel, 1948*).

Site of Chondrogenesis	Embryonic Age at Beginning of Chondrogenesis (days)	Duration of Chondrogenesis (days)
Meckel's cartilage (primitive mandible)	5.5	5.5
Branchial skeleton	5.5	5.5
Thoracic vertebrae	5.5	5.5
Lumbar vertebrae	5.5	5.5
Extremities	5.5	5.5
Orbital region	6.0	5.0
Trabeculae and parachordals (anterior part of basis cranii)	6.5	4.0
Pelvic and pectoral girdles	7.0	3.5
Caudal vertebrae	7.5	4.5+
Ribs	8.5	2.0

The cells, or chondroblasts, in the center of the cell mass (or, in a long bone rudiment, in the region corresponding to the middle of the future shaft, or diaphysis) now flatten slightly and become oriented transverse to the long axis of the cellular aggregation (Fig. 340-A). The mitochondria again lengthen into filaments, and the Golgi apparatus becomes smaller and begins to break up (*Weel, 1948*).

Very soon, the chondroblasts are separated by narrow intercellular spaces containing the first traces of cartilage matrix (*Fell, 1925*). The cells, as seen in the interior of the articulare are shown by the electron microscope to contain fine single filaments oriented roughly parallel to the short axis of each cell. Unlike the cytoplasmic processes of the cells, these intracellular



filaments do not seem to be in continuity with the fibrils of the matrix (Jackson, 1954).

The outermost cells of the mass align themselves parallel with the surface to form a rudimentary perichondrium. This stage is exemplified by the tibia of the 4.5-day chick embryo (Fell, 1925) and by the articulare of the 6-day

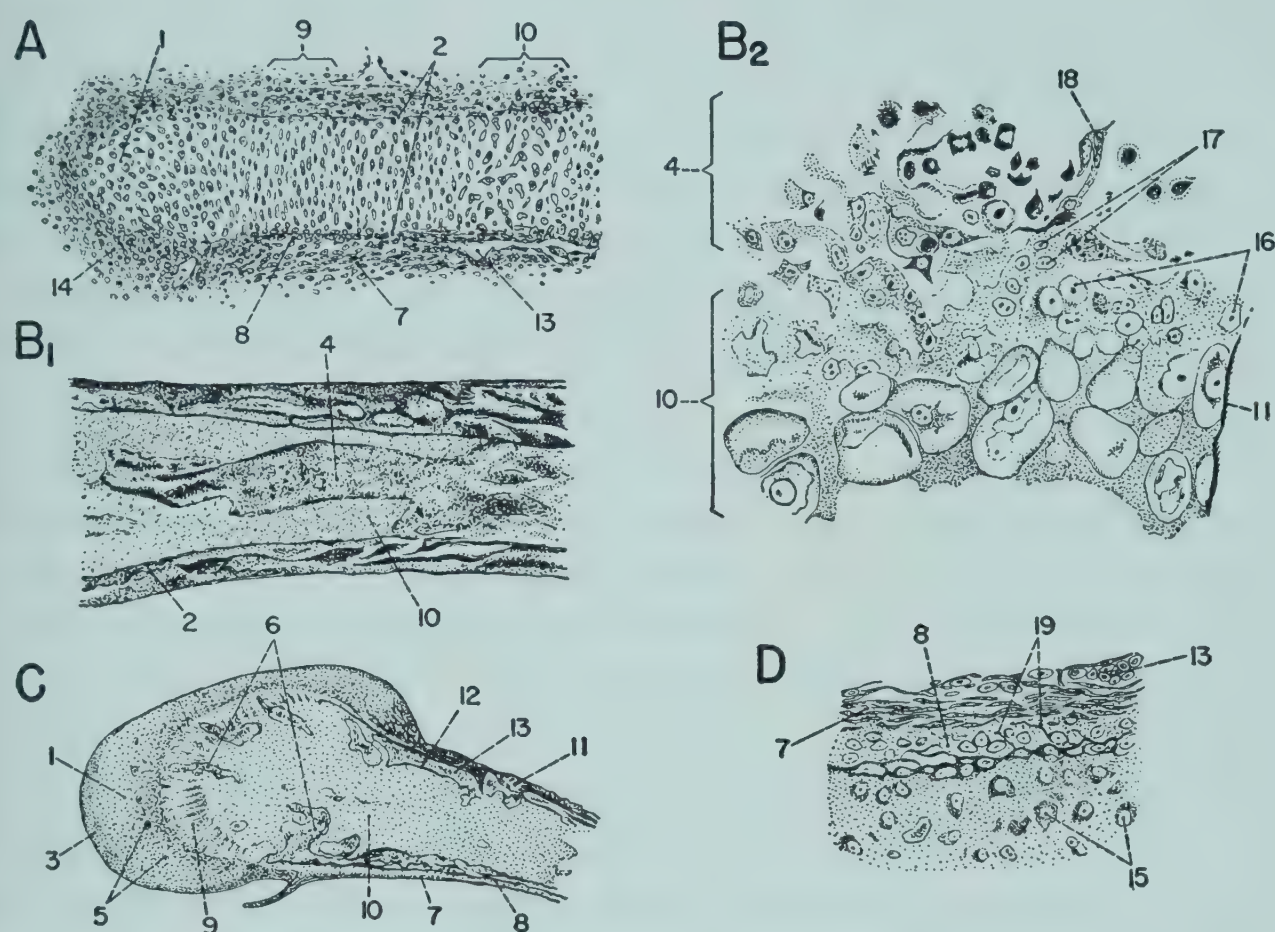


Fig. 340. Various stages in the histogenesis of the long bones in the chick embryo. (Redrawn with modifications after Fell, 1925.)

A, longitudinal section through the tibia of a 4.5-day embryo, showing appearance of three cellular zones in the cartilage and the formation of a cylinder of periosteal bone; B<sub>1</sub>, longitudinal section of the tibiotarsus of a 14-day embryo, showing resorption of cartilage and excavation of the marrow cavity; B<sub>2</sub>, detail of the zone of degeneration in the femur of a 14-day embryo; C, longitudinal section of the condyle and distal end of a day-old chick's femur; D, early formation of periosteal bone in a section from the middle portion of a phalanx from a 9.5-day embryo.

1, epiphysis; 2, central osseous cylinder; 3, articular cartilage; 4, marrow; 5, marrow cavities of epiphysis; 6, marrow cavities of diaphysis; 7, fibrous layer of periosteum; 8, osteoblastic layer of periosteum; 9, zone of flattened cells; 10, zone of hypertrophied cells; 11, periosteal bone; 12, endochondral bone; 13, blood vessel; 14, mesenchyme; 15, hypertrophied cells; 16, disintegrating chondroblast; 17, disintegrating cells of marrow reticulum; 18, normal cells of invading marrow; 19, osseous lamellae.

embryo (Fell and Robison, 1930). The electron microscope reveals that the cytoplasmic portions of the perichondral cells in the long bone rudiments of the 5-day embryo are prolonged into fine processes intertwined with those of adjacent cells; apparently no cell membrane is present. In the articulare, that is, in the proximal part of Meckel's cartilage, the perichondral cells are differentiating into chondroblasts by apposition, and



newly formed chondrogenic cells contain intracytoplasmic filaments paralleling the long axis of the cells (*Jackson, 1954*). More radioactive sulfur is fixed by the perichondrium than by the surrounding tissue (*Amprino, 1955a*).

The transverse orientation and flattening of the chondroblasts and the appearance of intercellular matrix proceed outward from the center of a bone primordium; in a developing long bone, progress is from the mid-diaphysial region distalward toward the ends, or epiphyses, which remain separate from the diaphysis until after hatching. The future boundary between the diaphysis and each epiphysis is indicated by the alignment of the cells in a curve convex to the epiphysis (cf. Fig. 340-A). The early epiphyseal region is composed of stellate cells embedded in matrix but is not sharply demarcated from the surrounding undifferentiated tissue (*Fell, 1925*).

Beginning in the center of a bone rudiment, the flattened chondroblasts cease dividing and start to enlarge; at the same time, they become round or polyhedral in shape (cf. Fig. 340-A). Eventually, they attain about three times their original size, the increase being relatively greater in their cytoplasmic than in their nuclear portions (*Fell, 1925; Jackson, 1954*). The cytoplasm of some cells now contain long, fine, parallel filaments made visible by the electron microscope (*Jackson, 1954*). As the cells grow larger, the intercellular partitions become broader. The process of cellular hypertrophy begins in the chick's long bone primordia on the sixth or seventh day of incubation (*Fell and Robison, 1934*) but does not occur in the articulare until the fourteenth or fifteenth day (*Fell and Robison, 1930; Erdmann, 1940*).

The initiation of cellular hypertrophy in the middle of a bone rudiment is accompanied by changes elsewhere (*Fell, 1925*). The first signs of ossification appear beneath the perichondrium surrounding the hypertrophic zone; hence the hypertrophic zone represents the ossification center. The perichondrium, now more distinct, extends itself distally in both directions to cover the region of flattened cells, which are actively dividing and constitute a zone of proliferation (*Brachet, 1893*). In the epiphysis of a long bone, the matrix becomes of equal density throughout and the shape of the cells changes from stellate to round or polyhedral. On the external surface of the epiphysis, the cells become slightly flattened and thus presage the differentiation of the articular cartilage. By the middle of incubation, the epiphysis is covered with three or four layers of fusiform cells which are continuous at the epiphyseodiaphysial junction with the perichondrium of the diaphysis (*Brachet, 1893; Fell, 1925*).

Cellular hypertrophy, accompanied and followed by cellular degeneration, spreads distally as new cells are added at the ends of the hypertrophic zone, which is thus lengthened while the proliferative zones continually



move to a relatively more distal position. On the chick's sixth or seventh day of incubation, the hypertrophic zone occupies about one third of the total length of a long bone rudiment (*Fell, 1932*); on the tenth day, it constitutes about half the diaphysis (*Fell, 1925*). Erosion of the cartilage begins at this time (*Fell and Robison, 1934*) in the region where the cells first become hypertrophic and where they are now extensively degenerated.

Once it has begun in the rudiment of a long bone, the differentiation of cartilage continues almost normally if the entire bone rudiment is explanted and grown as a culture. Although forming more slowly than *in vivo*, all the various zones appear; erosion of the cartilage and its invasion by marrow tissue do not occur, however. The addition of insulin to the medium retards the differentiation of the diaphysial cartilage, for unexplained reasons; the proliferative zone is either poorly defined or absent, and the enlarged cells never pass beyond the early stage of hypertrophy (*Chen, 1954*). Thyroxine, on the other hand, accelerates the maturation of cartilage *in vitro*, the stimulation being inversely proportional to the age of the bone rudiment at the time of explantation (*Fell and Mellanby, 1955*). Vitamin A, in concentrations corresponding to hypervitaminotic levels, causes softening and shrinkage of the cartilage matrix of the diaphysis (and eventually of the epiphyses) but does not produce degeneration of chondroblasts (*Fell and Mellanby, 1952*).

At the time of hatching, hypertrophy of cartilage cells in a typical bone has spread to the ends of the diaphysis, but the cartilage of the middle one half to two thirds of the shaft has been eroded and replaced by marrow. Between the enlarged cells of the metaphyses (the extremities of the diaphysis), there is less matrix than in the remaining four fifths of the residual cartilage (*Stricht, 1890*). Each of the proliferative zones forms a narrow belt between, and sharply demarcated from, the epiphysis and the diaphysis (Fig. 340-C). The cells of the proliferative zone are arranged in linear columns (*Stricht, 1890*) paralleling the long axis of the bone, and many of them are undergoing degeneration. The epiphysis, which has continued to become more sharply delimited during incubation, is now a flattened cap of cartilage (cf. Fig. 340-C) with an extremely dense matrix (*Fell, 1925*); the cells are barely beginning to enlarge (*Martin, 1954*). The uptake of radioactive sulfur is always lower in the epiphysis than in the diaphysis, and the difference is now very conspicuous (*Amprino, 1955a*). Over the articular surface of the epiphysis, the flattened cells, recognizable as the developing articular cartilage, have become more compressed and form a distinct layer (cf. Fig. 340-C); the cells are interspersed with thick bundles of white fibers. Medially, the fibrous articular cartilage grades imperceptibly into the hyaline cartilage of the epiphysis (*Fell, 1925*). Observation of the 3-day-old turkey, *Meleagris gallopavo* (*Stricht, 1890*), indi-



cates that the morphology of the articular and epiphyseal regions is much the same as in the newly hatched chick.

In birds, the cartilage of the long bones does not become calcified at the time it hypertrophies, as it does in mammals; apparently, the calcifying mechanism is incomplete and develops slowly (*Fell and Robison, 1934*). The first signs of calcification are seen in the long bones of the chick on the fourteenth or fifteenth day of incubation, in the form of small calcified areas situated immediately beneath the periosteal bone near the extremities of the diaphysis. On the eighteenth or nineteenth day, the residuum of cartilage that surrounds the distal ends of the marrow cavity has become impregnated with calcium salts for a short distance inward from its surface (*Brachet, 1893; Fell and Robison, 1934*). Three or four days after hatching, calcification has spread into the metaphyseal regions, in both the chick (*Fell and Robison, 1934*) and the turkey, *Meleagris gallopavo* (*Stricht, 1890*), but still does not extend as far as the proliferative zone.

On the other hand, the synthesis of phosphatase (which is involved in the process of calcification) is already taking place in the cartilage of the long bone rudiments on the chick's ninth day of development and probably begins as soon as the chondroblasts enlarge (*Fell and Robison, 1934*). The association between phosphatase production and hypertrophy of the chondroblasts is indicated by the lack of phosphatase activity in limb buds explanted to culture media while still mesenchymatous and in which chondrogenesis stops at the stage of small-celled cartilage (*Fell and Robison, 1929*).

**Structure of the cartilage matrix.** Under the optical microscope, the early cartilage matrix appears as a delicate film over the surfaces of the chondroblasts, including their processes (*Fell, 1925*). By means of the electron microscope, it can be seen that the intercellular substance is a loose, irregular meshwork of indistinct material (*Jackson, 1954*). As the cells begin to increase in size, they separate, and the matrix between them is at first pulled out into a thin, perforated sheet; then, upon the addition of more material, it becomes progressively denser, and the intercellular partitions grow wider (*Fell, 1925*). The electron microscope shows that the matrix now consists of a meshwork of well-defined fibrils which, however, lack the cross-striations characteristic of mature collagen fibrils. The matrix eventually grows very compact, its fibrils become oriented almost parallel to one another, and thickened bundles of fibers appear. Immediately surrounding each cell is a region, composed of fibrils embedded in an amorphous material, that gradually develops into a fibrous boundary, the capsule of the cartilage cell. In the articulare, the matrix is more diffuse than in the long bones (*Jackson, 1954*), which develop more rapidly.

About midway through incubation, short, tapering fibrils with the cross striations of mature collagen appear in the matrix; they have been observed



in the distal (metaphyseal) portions of the diaphysis of the 11-day chick embryo's tibia, embedded among the fine, unbanded fibrils which form the bulk of embryonic cartilage and which are also present in adult cartilage. The unbanded fibrils differ from collagen in that they are attacked by trypsin (*Martin, 1953, 1954*).

In epiphyseal and articular regions of a long bone, regions that are still unossified at the end of incubation, mature collagen fibers do not appear during embryonic life. In the optical microscope, faint bundles of fibers can be seen between flattened cells in the early cartilage matrix of these parts of the chick embryo's tibia, but the electron microscope reveals that the fibers are accumulations of bundles made up of fine, unbanded, apparently structureless fibrils, and that the matrix consists of a dense feltwork of such fibrils. Approximately at the midpoint of incubation, the fibrils are even more intricately interwoven and show no tendency to form parallel bundles or capsules around the cartilage cells of the epiphysis. A few coarser fibers with periodic cross-striations of narrower width than those of mature collagen fibers are now present in the epiphysis but not in the articular region of the tibia. During the last week of the chick's incubation period, the fibrous component of the epiphysis, very conspicuous in the light microscope, is resolved by the electron microscope into oriented bundles of nonstriated fibrils. The articular perichondrium consists almost entirely of banded fibers, some of which extend into the matrix of the underlying cartilage; but the periodicity of the striations is not that of mature collagen. Within 7 to 21 days after hatching, the immature banded fibers are replaced by mature collagen in the articular and epiphyseal regions, and mature and immature fibers appear in the narrow remnant of the zone of flattened cells, where, however, cells and cell debris predominate (*Martin, 1953, 1954*).

The reasons for the early differentiation of mature collagen in the cartilage matrix of the diaphysial region and its later differentiation elsewhere are not known, but it has been proposed that there is some relationship between the rate of ossification and the type of fibril present in the matrix (*Martin, 1953, 1954*). The origin of the fine fibrils of which embryonic cartilage is largely composed is equally uncertain, but the evidence suggests that the intracytoplasmic filaments in the hypertrophic chondroblasts are probably concerned in their genesis (*Jackson, 1954*).

**Perichondral Ossification.** Perichondral ossification begins in the same region as chondrogenesis, that is, in the central or mid-diaphysial section of a bone rudiment. As the chondroblasts start to enlarge, the perichondrium covering the zone of hypertrophy differentiates into an outer layer of fibroblasts and an inner layer of osteoblasts (cf. Fig. 340-A). In a long bone, the formation of the fibroblastic and osteoblastic layers, keeping pace with or preceding the extension of the hypertrophic zone, progresses distal-



ward toward the epiphyses and ceases at the boundaries between the epiphyses and the diaphysis (*Fell, 1925*).

In the chick, the cells of the fibroblastic layer are fusiform in shape, each with an elongated nucleus, a small reticular Golgi apparatus, and mitochondria scattered throughout the cytoplasm, including the terminal filamentous processes (*Fell, 1925*). One or two intracytoplasmic filaments, visible in the electron microscope, parallel the long axis of the nucleus and extend into the cytoplasmic processes (*Jackson, 1954*). The fibroblastic layer is at first diffuse peripherally but gradually becomes more condensed and more sharply defined, although it remains continuous with the intramuscular connective tissue. It is pierced by vascular clefts, which soon give rise to small blood vessels. During the course of development, the fibroblastic layer is strengthened by the formation of white fibrous tissue (*Fell, 1925*). By means of the electron microscope, it can be seen that the cells are connected by extracellular fibrils, some fine and some thicker and undulating. Those of the latter type seem to emerge from the cytoplasmic processes or from the periphery of the cells and appear to be composed of finer parallel elements (*Jackson, 1954*). At the boundary of the epiphysis and the diaphysis, the inner layers of the fibroblastic coat merge their fibers with those of the cartilage matrix of the epiphysis, while the outer layers become continuous with the fibrous tissue of the articular surface (*Fell, 1925*).

The osteoblasts which are held responsible for the deposition of bone, are very slightly flattened, with their long axes parallel with the circumference of the cartilaginous rudiment of a long bone. They are arranged in layers, and developing blood vessels are present between their closely interwoven cytoplasmic processes. Each osteoblast contains a spherical, somewhat eccentric nucleus, two small nucleoli, a reticular Golgi apparatus, filamentous mitochondria, and numerous cytoplasmic granules about 0.7 microns in diameter (*Fell, 1925; Jackson, 1954; Jackson and Smith, 1955*). Tests indicate that the granules contain alkaline phosphatase and possibly dehydrogenase systems (*Jackson and Smith, 1955*). When viewed in the electron microscope, the osteoblasts are seen to be filled with long, parallel filaments, which are associated with the mitochondria and the cytoplasmic granules and are arranged in layers running transverse to the long axis of the bone rudiment (*Jackson, 1954; Jackson and Smith, 1955*).

Soon after the perichondrium acquires its two-layered structure, delicate, undulating lamellae appear beneath the osteoblasts. They parallel the long axis of the cartilage and are composed of fine interwoven fibers cemented together by an amorphous material which increases in quantity as development proceeds (*Fell, 1925*). The electron microscope reveals that some of the fibrils of this osteoid tissue are indistinguishable from the intracytoplasmic filaments of the osteoblasts, with which they seem to be continuous.



The fibrils nearest the cartilage are parallel to the surface of the lamellae but appear to originate from the cartilage matrix (*Jackson, 1954*). The perichondrium, however, is responsible for bone formation at early stages; this capacity is exhibited by perichondrium removed from the 6-day chick embryo and grown *in vitro* in the absence of cartilage and a blood and nerve supply (*Fell, 1932*).

The formation of fibrous lamellae proceeds distalward and at the same time begins among the more peripheral layers of osteoblasts (Fig. 340-D). The lamellae grow larger and thicker, come into contact with each other, and fuse to form a thin cylindrical sheath of osteoid tissue beneath the perichondrium (*Fell, 1925*). In the chick, the osteoid sheath covers the middle one third of the rudimentary long bones by the sixth day of incubation, but it is not apparent in the articulare until the fourteenth day (*Fell and Robison, 1930*), when hypertrophy of the chondroblasts is first seen.

Calcification of the osteoid sheath begins on the chick embryo's seventh or eighth day. The calcified region covers about one fifth of the total length of a long bone by the end of the eighth day (*Fell and Robison, 1934*).

During the formation of the bony cylinder, many osteoblasts are included within its substance and thus become bone cells, or osteocytes. The nuclei of the osteocytes are more eccentric than those of the osteoblasts, and their cytoplasmic components contain some filaments, made visible by the electron microscope (*Jackson, 1954*), and are prolonged into fine, branching processes which extend into the intercellular substance (*Fell, 1925*).

The bony cylinder increases in thickness as additional material is deposited on its outer surface and as the more external lamellae fuse with it peripherally; erosion of its inner surface, resulting in a widening of the marrow cavity, does not occur during embryonic life. The osseous tissue is always thickest around the mid-portion of a developing long bone. This part of the diaphysis, where division of the chondroblasts long since ceased, is soon surpassed in diameter by the cartilaginous distal parts of the shaft, which continue to broaden. The fibroblastic layer of the perichondrium (or periosteum, as it may now be called) is pulled loose from the mid-diaphysis as the ends of the shaft enlarge. The osteoblasts become scattered in the space between the fibroblastic layer and the bone and are broken up into intervascular groups by the vessels of a developing vascular plexus (*Fell, 1925*). By the chick's tenth day of incubation (*Fell, 1932*), bone is beginning to be deposited in the form of short, irregular trabeculae more or less transverse to the central cylinder and fused with it at their inner ends. These trabeculae grow longer as the space between the fibroblastic layer and the bone tube increases. Osteoblasts situated between the fibroblastic layer and the vascular plexus now begin to produce a second series of bony lamellae, concentric with the inner osseous cylinder (*Fell, 1925*).

The bony trabeculae in the mid-diaphysial region become connected and,



instead of running transversely from the inner cylinder to the outer lamellae, incline themselves so as to be almost parallel with the long axis of the bone (Fig. 340-B<sub>1</sub>). The connections between the intervascular trabeculae continue to increase in size and number until the blood vessels are completely enclosed in osseous tubes lined with endosteal osteoblasts; these tubes form the Haversian systems characteristic of bone (*Fell, 1925*).

Both periosteal and endosteal osteoblasts take up radioactive sulfate within a few hours after its administration, whereas organic bone matrix does not. A few days afterward, however, most of the sulfate is found in the bone substance (and especially in the inner, first-formed layers) rather than in the osteoblasts (*Amprino, 1955a*).

On the chick's twelfth day of development, calcification of the osteoid sheath has progressed almost as far as the epiphyses of the long bones (*Fell and Robison, 1934*). Phosphatase activity in the developing bones closely parallels calcification. Phosphatase is not found in the osteoid tissues on the sixth day but is present on the ninth day, and its activity increases thereafter until the end of incubation. The most rapid rise occurs between the fifteenth and eighteenth days. The ratio of phosphatase to the dry weight of the long bones (and of other cartilage bones also, such as the quadrate bone) is at a maximum on the twelfth or thirteenth day (*Fell and Robison, 1929, 1930*). The synthesis of the enzyme in the tissue itself is indicated by the observation that phosphatase activity in cultured explants of 6-day chick femoral and quadrate bones becomes continually greater with the passage of time (*Fell and Robison, 1930*). The fibrous layer of the periosteum apparently plays no role in calcification, for cultured fragments of this tissue evince almost no phosphatase activity and do not calcify when immersed in calcifying solutions (*Fell and Robison, 1934*). Phosphatase does not appear, either *in vivo* or *in vitro*, in the distal part of Meckel's cartilage, which never ossifies (*Fell and Robison, 1930*).

The role of phosphatase in ossification is not entirely clear. It is believed that phosphatase hydrolyzes phosphoric esters so as to liberate phosphate ions, which then combine with calcium so as to deposit a form of calcium phosphate in the bones. Another mechanism, however, appears to be involved in the calcification of bone. The substrate for phosphatase is perhaps phosphopyruvate, the product of the degradation of glycogen by a system of enzymes—specifically, phosphorylase, dehydrogenase, and enolase. Calcification of cultured metatarsals and fibulas from 9- to 10-day chick embryos is inhibited in the presence of high concentrations of sodium fluoride (*Paff and Boyd, 1952*), which is known to inhibit enolase, the enzyme immediately involved in producing phosphopyruvate. Alizarin red S similarly halts calcification (*Paff, Angulo, and Eksterowicz, 1951*) without affecting phosphatase activity (*Paff and Sallman, 1951*). More calcium is deposited in cultured femurs from the 6- to 7-day embryo at pH levels of 7.0 to 7.4



than at more alkaline levels (*Yoshitomi, 1935; Paff, 1948*), which greatly favor the activity of phosphatase. The addition of phosphatase to the medium does not hasten ossification (*Cappellin, 1949, 1950*). Sugars, however, inhibit calcification (*Yoshitomi, 1935*), no doubt through their effect on phosphorylase.

**Formation of marrow cavity.** In a developing long bone of the chick, the cartilage matrix in the future diaphysis begins to be eroded as soon as the degeneration of the chondroblasts and the formation of periosteal bone are fairly well advanced. Erosion starts when the zone of hypertrophied cells occupies about half of the cartilaginous diaphysis, or about at the time that the second, more peripheral series of bony lamellae first appears beneath the periosteum (*Fell, 1925*). Destruction of the cartilage leads to the formation of the marrow cavity and is not preceded by calcification or accompanied by endochondral ossification, except in the epiphyses and the extremities of the diaphysis. The marrow cavity becomes filled with marrow tissue, which thus replaces the cartilage in the interior of the bony cylinder.

Like chondrification and perichondral ossification, erosion begins in the middle of the diaphysis. Here blood vessels proceeding from the vascular plexus of the cancellous bone erupt through the inner osseous cylinder. Accompanied by osteoblasts and connective tissue, they invade the cartilage. The matrix dissolves as they advance and the chondroblasts are liberated. Through the growth and branching of the vessels, the cartilage is honeycombed with excavations. These gradually enlarge and become confluent into a single, large cavity within the osseous cylinder (*Fell, 1925*).

This cavity becomes occupied by abundant anastomosed arterial and venous capillaries and a few larger, longitudinal arterial and venous vessels. The intervascular spaces are filled with marrow tissue in increasing quantity. Nongranular lymphoid elements and eosinophilic and other granular leucocytes appear in the intervascular connective tissue; granular cells and erythroblasts occur both in the marrow cavity and in the cancellous bone (see Chapter 8). Adipose cells are also present. The multinucleated giant cells known as chondroclasts or osteoclasts, which are always found in developing bone, are seen in the marrow and along the surfaces of the periosteal bone and the cartilage (*Brachet, 1893; Fell, 1925*).

The disappearance of the cartilaginous matrix progresses distalward (cf. Fig. 340-B<sub>1</sub>) with the distal extension and proliferation of the blood vessels already present; the invasion of new vessels near the extremities of the bony cylinder is infrequent (*Fell, 1925*). The blood vessels are accompanied by proliferating marrow tissue. At the border of the disintegrating cartilage is a zone of tissue of a composite nature, where the invading marrow cells become mingled with the chondroblasts as the latter are liberated from the matrix. Brachet (1893) regarded this zone as one of regeneration, in which



the chondroblasts become transformed into marrow cells, including osteoblasts; Stricht (1890) held a similar view. Fell (1925), however, presented evidence that this region is, on the contrary, a zone of degeneration (Fig. 340-B<sub>2</sub>). According to her observations, the newly liberated chondroblasts, already highly degenerate, briefly accumulate on the surface of the matrix before breaking down completely into protoplasmic debris; Strelzoff (1873) also stated that the chondroblasts disappear. Between the liberated chondroblasts and the normal marrow tissue are numerous disintegrating marrow connective tissue cells and degenerating osteoblasts.

Between the chick's tenth incubation day, when erosion begins, and the fifteenth day, the cartilage of the middle one third of the diaphysis is completely removed (*Fell and Robison, 1934*). Beginning on the thirteenth day, histiocytes, identifiable by their inclusions of injected trypan blue, appear in the marrow tissue and vascular endothelium in this part of the medullary cavity. It has been claimed that the transformation of histiocytes into osteoblasts can be seen on and after the fifteenth day (*Cappellin, 1951*). Erosion now becomes more rapid around the circumference of the cartilage than at its center, following the outgrowth of long, distally directed vascular processes which occur at fairly regular intervals immediately beneath the bony cylinder (Fig. 340-C). As a result, the still uneroded cartilage at either end of the diaphysis tends to assume the form of a cone whose apex extends into the marrow cavity (*Stricht, 1890; Brachet, 1893; Fell, 1925*). By the seventeenth or eighteenth day, about two thirds of the hypertrophic cartilage has been removed and largely replaced by marrow (*Fell and Robison, 1929*). In the pigeon (*Columba livia*), however, the apexes of the cartilaginous cones are still close together in the middle of the shaft (for example, of the humerus) 4 days after hatching (*Parsons, 1905*).

The manner in which erosion of cartilage is accomplished is uncertain. It is possible that the multinucleated giant cells (osteoclasts) assist in breaking down the cartilage matrix, but they are not sufficiently numerous in the beginning to be the sole agents of destruction (*Brachet, 1893; Fell, 1925*). The walls of the blood vessels—which *Brachet (1893)* described as the source of the osteoclasts, although they probably represent fused osteoblasts, connective tissue cells (*Fell, 1925*), or wandering cells (*Hancox, 1949a, 1949b*)—appear to play a prominent part in the resorption of cartilage during late stages of endochondral ossification, when the blood vessels are pressed against the matrix and fill the ruptured capsules. In the earlier stages, however, the blood vessels are separated from the cartilage by the zone of degeneration, as previously noted; furthermore, when ossification occurs in cultured explants of cartilage from 8-day chick embryonic long bones, the cartilage is eroded by connective tissue cells in the absence of ingrowing blood vessels (*Fell, 1928b*). In the opinion of *Fell (1925)*,



it is likely that all or most of the elements of the marrow exert a chondrolytic action. It is possible that hyaluronidase may play some role in the removal of cartilage, since chondroitin-sulfuric acid is known to be a substrate for this enzyme, and since the basophilia of chick embryo femurs treated with hyaluronidase *in vitro* is reduced (*Paff and Seifter, 1950*).

**Endochondral Ossification.** In birds, endochondral ossification is fairly extensive in the vertebrae and some other short bones but occurs only in the epiphyses and the distal one sixth of the long bones. It begins late in the incubation period, or even after its close, and is not completed until some time after hatching (*Fell, 1925*). It is always preceded by partial calcification of the cartilage composing the rudiment of a bone.

In long bones, the cone-shaped remnants of cartilage are replaced by bone formed endochondrally. When the marrow cavity begins to extend distally in the form of longitudinal excavations located around the cone peripherally, the osteoblasts within these excavations align themselves in the calcified surface of the cartilaginous cone (*Brachet, 1893*), often occupying the interior of ruptured capsules (*Jackson, 1954*). In these distal extensions of the marrow cavity, the liberated chondroblasts are in less advanced stages of degeneration than in the mid-diaphysial regions, and the zone of degeneration is composed largely of osteoblasts or is absent entirely (*Fell, 1925*). At the extreme distal end of the marrow cavity, irregular continuations of the medullary space penetrate medialward into the base of the cone, where the cartilage is also calcified to a certain extent. In the 3-day-old hatched chick or turkey (*Meleagris gallopavo*), the cone is separated from the proliferative zone and the epiphysis by a region that is honeycombed with branching marrow spaces lined with osteoblasts and the first traces of bone (*Stricht, 1890; Brachet, 1893*). Later, the bony linings, although frequently broken through and destroyed, form thin and largely incomplete trabeculae, which are increasingly numerous toward the end of the diaphysis. Through the confluence of longitudinal marrow spaces and transverse connecting excavations, the removal of the cone is accomplished (*Fell, 1925*) about 10 days after the chick hatches (*Brachet, 1893*); the destruction of the osseous walls of the marrow cavities is more extensive in this region than more distally, but the bony trabeculae that remain are thicker (*Fell, 1925*).

The epiphyses of the long bones ossify, but not until after hatching. Capillaries are present in the epiphyses on the chick's tenth day (*Cappellin, 1951*), and, by the end of incubation, marrow cavities have formed in the epiphyses independently of those in the diaphysis. Apparently, osteoblasts are not present at first in the intervacular tissue (*Fell, 1925*); histiocytes, however, are present between the zones of ossification on the seventeenth day (*Cappellin, 1951*). Shortly before hatching and in early postembryonic life, vascular processes penetrate into the epiphyses from the diaphysis. The



epiphyses then ossify as continuations of the diaphysis (*Parsons, 1905; Fell, 1925*).

Endochondral ossification takes place in the vertebrae and the cartilage bones of the skull during the chick's last 7 to 9 days of incubation. Only part of the cartilage matrix is calcified before it is resorbed and replaced by osseous and marrow tissue, in much the same manner as described above (*Amprino, 1955a*). Histiocytes become visible in the medullary spaces of the vertebrae on the twelfth day of development, and on the fifteenth day, granulopedy is exhibited by cells adherent to osseous lamellae, that is, in the position of osteoblasts (*Cappellin, 1951*).

### The Axial Skeleton

The axial skeleton consists of vertebrae, the ribs, and the skull, including the jaws and the hyobranchial skeleton. The sternum, with which the distal ends of the ribs articulate, is sometimes considered as part of the axial skeleton, but is generally more closely related to the appendicular skeleton. The axial skeleton is at first represented by the rodlike notochord, which occupies the midline, and by the paired somites into which the mesoderm on either side of the notochord becomes segmented. The vertebrae are derived from certain portions of the somites and form around the notochord and neural tube. The anterior end of the notochord and a few of the somites participate also in the development of the posterior part of the base of the skull. The remainder of the skull differentiates from unsegmented mesenchyme. The proximal portions of the ribs are of somitic origin; their distal portions probably arise from mesenchyme of the ventral and ventrolateral body wall. Some of the ribs are rudimentary and are attached to the vertebrae as processes.

### *The Primitive Axial Structures*

The establishment of the notochord and the somites may be considered as a preliminary phase in the development of the axial skeleton. Both the notochord and the somites undergo a certain amount of differentiation before the formation of skeletal elements actually begins.

**The Notochord.** The notochord or chorda is obviously the homologue of the permanent axial supporting rod of lower chordates. In birds, it is a transient embryonic structure, which eventually disappears entirely. Its function appears to be the passive one of providing an axis of differentiation, or, at best, may be the induction of vertebral cartilage.

The appearance of the notochord, known in its earliest, rudimentary form as the head-process, indicates that the regression of the primitive streak and the node has begun (see Chapter 3). As long as the primitive streak regresses, material is added to the posterior end of the notochord, which thus elongates in the midline in front of the streak until it extends



almost to the tip of the tail; its anterior end lies at the level of the future hypophysis.

The head-process appears in the chick embryo after about 16 hours of incubation. It is a rather diffuse cellular aggregation, merging into the pre-axial mesoderm anteriorly, fused with the endoderm below, and closely adherent to the ectoderm above (Rex, 1897; Adelmann, 1922; Kuhlenbeck,

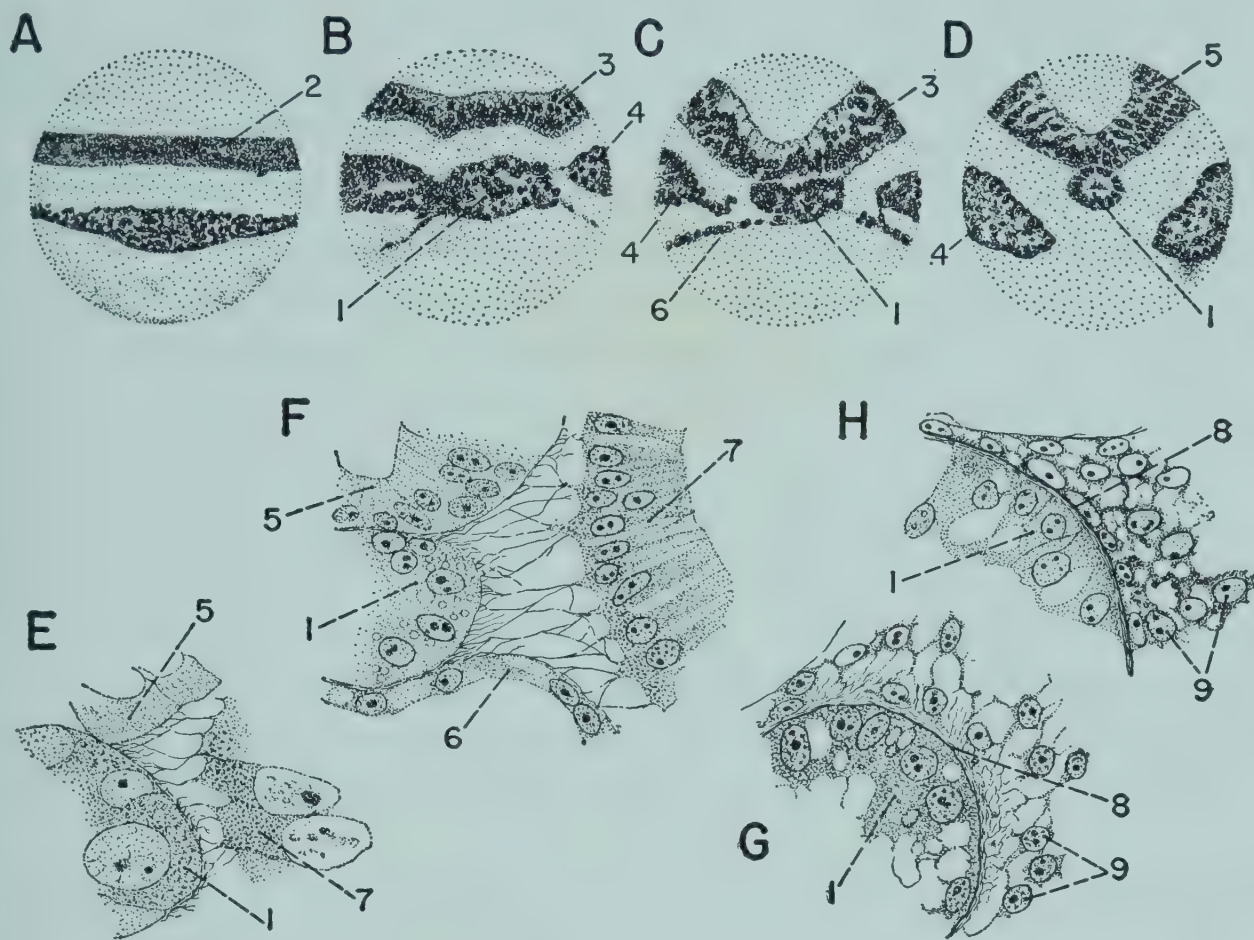


Fig. 341. Process of condensation and separation of the notochordal tissue in the chick and the duck (*Anas platyrhynchos*) from the surrounding mesoblast. (Redrawn with modifications A to D, after Kuhlenbeck, 1930; E to H, after Held, 1921.)

A, region of head-process in a 24-hour duck embryo ( $\times 25$ ); B, differentiation of the chorda anlage in a 24-hour chick embryo ( $\times 10$ ); C, same stage, when the chorda is independent of the surrounding tissue ( $\times 10$ ); D, relationship of the neural tube, notochord, and somites in a 36-hour chick embryo ( $\times 10$ ); E, an 8-somite duck, when the cells of the notochord are not yet differentiated ( $\times 400$ ); F, a 24-somite duck, when the cells have assumed their typical shape, and the vacuoles are beginning to appear ( $\times 200$ ); G, a 60-hour duck embryo, when vacuolization is well advanced and mesenchyme cells from the somites are migrating around the notochord ( $\times 200$ ); H, a 3.5-day duck embryo, when the fibrillar notochordal sheath is apparent ( $\times 200$ ).

1, chorda cells; 2, presumptive neural tissue; 3, neural plate; 4, somite; 5, neural tube; 6, endoderm; 7, sclerotome; 8, tunica propria of notochord; 9, mesenchyme.

1930). In its thickest portion, the head-process of the 24-hour duck (*Anas platyrhynchos*) embryo consists of as many as ten or twelve layers of irregularly arranged cells (Kuhlenbeck, 1930). The transformation of the head-process into the notochord, through condensation and separation from the surrounding tissues (Fig. 341-A, B, C, and D), proceeds from posterior to anterior; it begins about at the time the first pair of somites forms and



is complete at the 7-somite stage, or shortly thereafter (*Adelmann, 1922*). In the 24-hour chick or 36-hour duck embryo, the notochord extends forward as a distinct entity from a point just in front of the node almost to the anterior intestinal portal; it has become independent of both endoderm and mesoderm and consists of two rows of cells, of which only the dorsal row is regularly aligned. In embryos about 12 hours older, the dorsal and ventral rows have each become arranged in a semicircle, so that a cross section of the chorda is a circle or oval made up of eight to ten wedge-shaped cells. A small central lumen soon appears (*Kuhlenbeck, 1930*). The individualization of the notochord rapidly progresses as far craniad as the prechordal mesodermal plate, to whose median portion, however, the anterior end of the chorda remains attached (*Rex, 1897; Adelmann, 1922; Kuhlenbeck, 1930*). With the development of the cranial flexure between the 15- and 30-somite stages, endoderm delaminates in a caudocraniad direction from the ventral surface of the prechordal plate (see Chapter 4). The dorsal portion of the prechordal plate in the form of a thin, ventrally curving strand of cells now connects the end of the notochord with the dorsal wall of Rathke's pouch (*Rex, 1897; Adelmann, 1922, 1926*). In the 31-somite chick embryo (*Adelmann, 1926*), as in the 30-somite (*Rex, 1897*) or 72-hour duck (*Anas platyrhynchos*) embryo (*Kuhlenbeck, 1930*), this strand of cells is continuous with the dorsal wall of the mesenchymal condensation (transverse commissure) connecting the bilateral premandibular head cavities, but it eventually disappears (*Adelmann, 1926*).

Meantime, the internal differentiation of the chorda has begun and, as at all subsequent stages, is most advanced approximately at the level of the transition from brain to spinal cord (that is, the future cervical region), from which level there is a gradual decrease in the degree of differentiation caudalward to the tail bud and craniad to the rostral end of the chorda (*Piiper, 1928; Kuhlenbeck, 1930*). As the result of mitotic activity, which is very marked during the differentiation of the notochord, the walls of the chorda have become several cell layers thick in the cervical region of the 72-hour duck (*Anas platyrhynchos*) embryo. Vacuoles have begun to appear in the innermost layers, and the chordal lumen has become wide and indistinctly delimited, probably because of the influx of protoplasm from liquefied cells. The process of vacuolization is illustrated in Fig. 341-E, F, G, and H. In the 90-hour duck, the chorda has acquired its definitive appearance of a "gelatinous body"; that is, its central portion has become a protoplasmic reticulum whose trabeculae enclose large vacuoles and whose nodal points are occupied by nuclei. The periphery is composed of a thin, nucleated syncytial layer containing no vacuoles; this layer is regarded as the chordal epithelium. In the 5-day duck embryo, the gelatinous body extends throughout the entire notochord with the exception of the portions at the caudal end of the embryo's body and in the tail bud, within



which the hollow notochord is attached to the tail gut by a strand of cells (the so-called hypochorda). Characteristic features of the fully differentiated notochord are numerous rod-shaped nuclei (possibly degenerated nuclei of nonvacuolated cells), seen in the 6-day chick embryo, and an axial strand of condensed trabeculae, seen in the 7-day chick embryo (Kuhlenbeck, 1930).

External to the limiting membrane of its epithelium, the notochord becomes surrounded by the perichordal sheath, which consists of an inner fibrillar membrane and an outer zone of flattened cells. The membranous component of the sheath is derived either from the fibrillar intercellular ground-substance that fills the various cavities of the early embryonic body (Baitsell, 1925) or from cell processes of mesenchymal elements adjacent to the notochord. The fibrous sheath can be seen developing in the 8- to 14-somite duck (*Anas platyrhynchos*) embryo (Held, 1921) and in the 36- to 48-hour chick embryo (Williams, 1942). It fuses so intimately with the peripheral notochordal cells that no line of demarcation can be seen (Williams, 1942). The cellular component of the sheath is added during the duck's fourth day of development (Kuhlenbeck, 1930; Held, 1921) and the gull's (*Larus canus*) second and third day (Piiper, 1928), and between the chick's forty-eighth and eightieth hours of incubation (Williams, 1942). Its cells are contributed by the mesoderm of the sclerotomes, the portions of the somites that are involved in the development of bony structures (Williams, 1942).

With the formation of the perichordal sheath, the notochord becomes an integral part of the developing axial skeleton. Whether it is an active or a passive part is not entirely clear. It may play only a mechanical role (Williams, 1942); however, there is considerable evidence that it can induce the formation of cartilage and is essential for the evolution of normal vertebral bodies, or centra (the heavy ventral portions of the vertebrae, as distinct from the portions surrounding the spinal cord). There is a tendency for cartilage to form about notochordal tissue in blastoderms grafted on the chorioallantois while in the definitive primitive streak to early somite stages (Willier and Rawles, 1931b); and experimental results indicate that the notochord, transplanted into a host embryo, can induce the differentiation of vertebral cartilage from host tissue. Also developing vertebral centra conform exactly to the shape of multiple or abnormal notochords (Watterson, Fowler, and Fowler, 1954). After extirpation of the notochord (without damage to the neural tube), vertebrae form around the neural tube; their dorsal regions are normal, but their ventral regions are reduced in size (Watterson, Fowler, and Fowler, 1954) and nonsegmented (Strudel, 1955a, 1955b).

Approximately on the chick's tenth day of incubation, the notochord begins to undergo retrogression, a process involving nuclear degeneration



and a decrease in the size of the vacuoles, which disappear by the fourteenth day. Twenty-four hours later, the small amount of remaining notochordal tissue has once more become cellular in structure and closely resembles cartilage (*Williams, 1942*).

**The Somites.** The division of the paraxial, the thickest portion of the mesoblast, into segments or pairs of somites is a prominent feature of avian as well as of all vertebrate ontogeny. The somites are blocks of tissue, rectangular in surface view, which flank the notochord. They contain the primordia of skeletal elements, part of the voluntary musculature, and a portion of the integument.

The somites appear in succession from anterior to posterior until they form a double row that extends, in birds, from the future occipital region almost to the end of the tail bud. Segmentation at more cranial levels, although recognizable in certain lower vertebrates, is suppressed, except for a vestige of the most anterior segment, the premandibular or (from its position anterior to the ear) the first pro-otic segment. In addition, two of the cranial segments behind the ear (postotic or metotic) are also rudimentary and transitory, and a number of the most caudal pairs of somites are resorbed in the course of development.

The first indication of mesodermal segmentation is the appearance of two approximately transverse furrows, one at each side of the (temporarily) posterior end of the notochord, that is, slightly in front of the anterior end of the primitive streak. In the chick embryo, these furrows are seen after about 21 or 22 hours of incubation (*Patterson, 1907*), sometimes earlier. They constitute the anterior boundaries of the first pair of persisting somites (*Platt, 1889; Patterson, 1907; Williams, 1910*). Longitudinal sections show that each furrow consists of a depression in the dorsal surface of the mesoderm and a corresponding indentation in the ventral surface. After about 24 hours of incubation (*Kupffer and Benecke, 1897*), but usually somewhat sooner (*Patterson, 1907*), a similar set of furrows indicates the posterior boundaries of the first somites, and additional paired intersomitic clefts begin to develop still more posteriorly (Fig. 342).

While the third pair of transverse furrows is forming, a longitudinal groove appears on either side of the notochord, parallel to it and a short distance away (*Williams, 1910*). This groove not only indicates the lateral boundaries of the first and second somites but extends posteriorly to divide the mesoderm on each side into a thin lateral portion, or lateral plate, and a thick medial portion, the somitic plate (paraxial, segmental, or vertebral plate). As the primitive streak recedes, the somitic plate elongates posteriorly while its anterior end is continually shortened by the differentiation of additional somites. When the coelom forms and separates the lateral mesoderm into an upper and a lower layer, a thin undivided strip of mesoderm,



the nephrotome or intermediate cell mass, remains between the somitic and lateral plates.

The first somites to appear on each side remain connected with one another until perhaps six or seven are present, when the clefts between them become complete fissures through the mesoderm. Thereafter, each successively more posterior somite forms in the unsegmented mesoderm before there is any indication of a furrow behind it and it has separated entirely from the somitic plate before another somite begins to develop (*Patterson, 1907*).

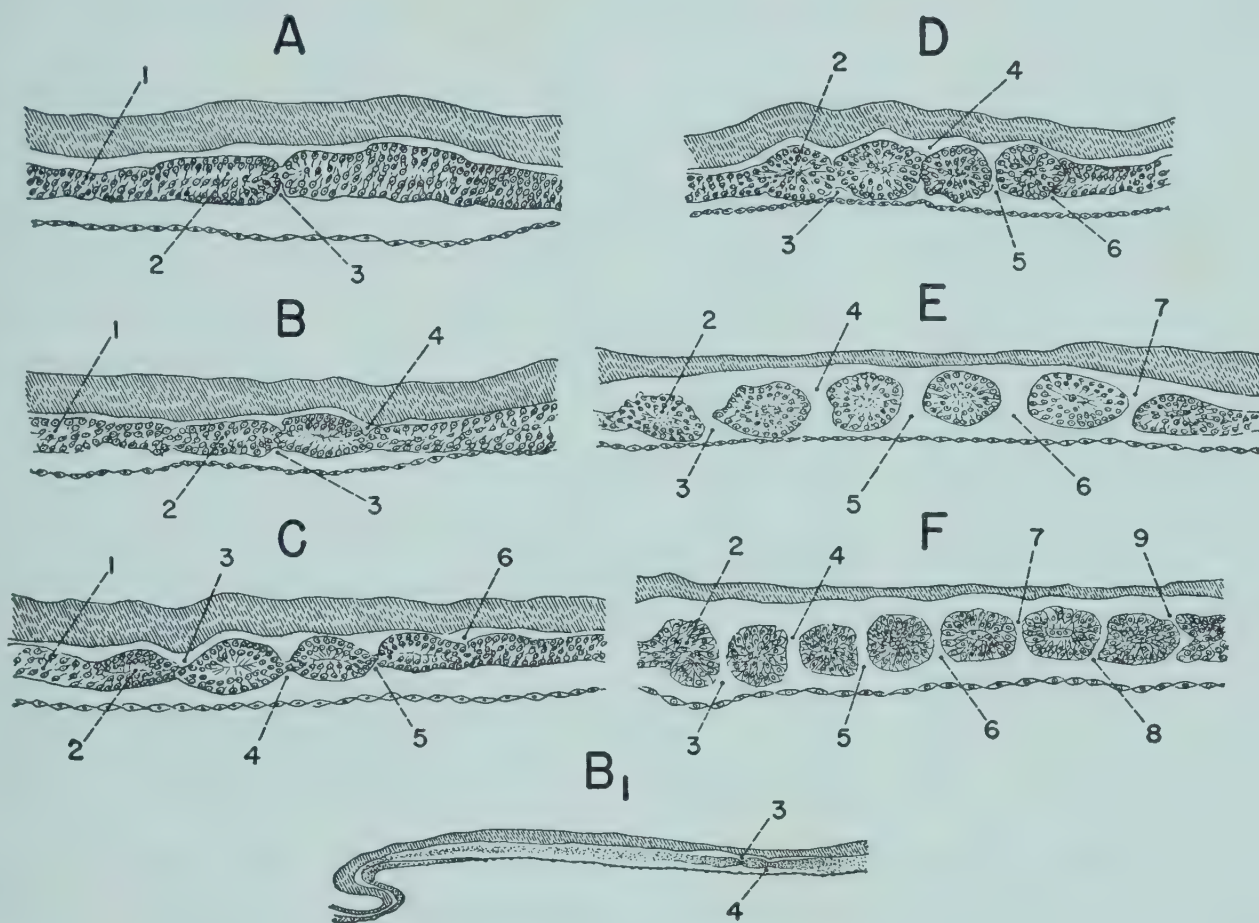


Fig. 342. A series of sagittal sections illustrating the segmentation of the somitic plate mesoderm in the chick. (Redrawn with modifications after *Patterson, 1907*.)

A, dorsal and ventral furrows of the first mesodermal cleft are formed and there are indications of the second and third clefts ( $\times 60$ ); B, region of the first somite ( $\times 60$ ); B<sub>1</sub>, relationship of the first and second clefts to the whole embryo ( $\times 30$ ); C, 3-somite embryo ( $\times 60$ ); D, 4-somite embryo ( $\times 60$ ); E, 6-somite embryo ( $\times 60$ ); F, 7-somite embryo ( $\times 60$ ).

1, somitic mesoderm; 2, rudimentary somite; 3 to 9, first through seventh mesodermic clefts, marking off the first six permanent somites.

While the first six or seven somites are differentiating behind the first intersomitic furrow, two temporary somites slowly make their appearance anterior to it, between the furrow and the middle of the otic placode (*Platt, 1889; Chiarugi, 1890; Jager, 1926; Beer and Barrington, 1934; Hinsch and Hamilton, 1955*). These are the first and second metotic somites. The second, or more posterior of the two, is almost normal in appearance. It differentiates from the anterior unsegmented mesoderm when approxi-



mately four or five somites are present (*Platt, 1889*). It begins to break down into mesenchyme at the 18-somite stage and disappear before the 30-somite stage (*Rex, 1905; Jager, 1926; Beer and Barrington, 1934*); the vagus nerve then appears at the same level. The first (more anterior) metotic somite forms later than the second and disappears earlier. It is rudimentary, consisting of only a few cells, and never separates completely from the second metotic somite behind it or from the unsegmented mesenchyme in front of it. It disappears between the 10- and 14-somite stages (*Rex, 1905; Beer and Barrington, 1934*). The disappearance of these two somites is illustrated in Fig. 343. Carbon-marking experiments indicate that the

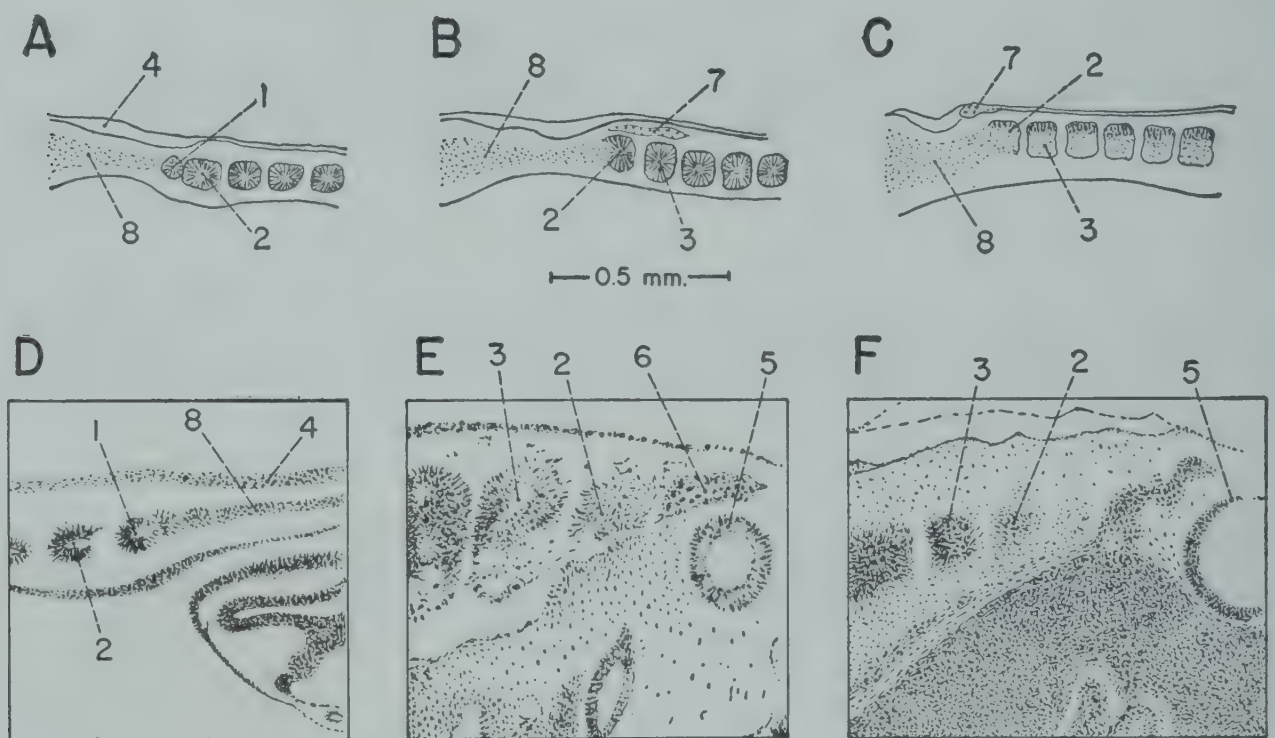


Fig. 343. Successive stages in the development of the metotic region in the duck embryo (*Anas platyrhynchos*) showing the disappearance of the anterior two metotic somites. (Redrawn with modifications after *Beer and Barrington, 1934*.)

A, reconstruction of sagittal section from the 7-somite stage ( $\times 25$ ); B, same, from the 14-somite stage ( $\times 25$ ); C, same, from the 18-somite stage ( $\times 25$ ); D, parasagittal section from the 10-somite stage (in scale); E, same, from the 23-somite stage (in scale); F, same, from the 39-somite stage (in scale).

1, 2, 3, first, second, and third metotic somites; 4, auditory placode; 5, auditory sac; 6, glossopharyngeal nerve; 7, neural crest; 8, mesenchyme.

second metotic somite forms head mesenchyme at a level between the ninth and tenth cranial nerves (*Hinsch and Hamilton, 1955*). The extremely transitory nature of the first two metotic somites accounts for the conclusion, reached by *Patterson (1907)* and *Hubbard (1908)*, that only the rudimentary somite forms anterior to the first transverse furrow.

In birds, then, the first pair of permanent somites, which always lies immediately behind the vagus nerves, is actually that of the third postotic or metotic segment. The first permanent somites also comprise the sixth segment of the entire series as is indicated by the temporary existence of the bilateral premandibular head cavities. These cavities are considered to



be the vestigial equivalents of the first pro-otic somites since in certain lower vertebrates they are associated with the development of the extrinsic eye muscles supplied by the oculomotor nerve. These head cavities have been recognized in a number of birds, including the duck, *Anas platyrhynchos* (Wijhe, 1886; Rex, 1897, 1901); the tern, *Sterna hirundo*; the lapwing, *Vanellus vanellus* (Wijhe, 1886); the gull, *Larus canus* (Wijhe, 1886; Rex, 1905); the robin, *Erithacus rubecula* (Adelmann, 1925, 1926); and the chick (Edgeworth, 1907; Adelmann, 1926). They form at the 22- or 23-somite stage (Rex, 1897, 1905; Adelmann, 1926) in the condensed lateral portions of the prechordal mesodermal plate (Adelmann, 1926) and are connected across the midline by its median portion, which may also become hollow (Edgeworth, 1907; Beer and Barrington, 1934). The cavities are obliterated by the 40-somite stage in the chick (Adelmann, 1926) and by the end of the fifth day in the duck, *Anas platyrhynchos* (Beer and Barrington, 1934), when the eye muscle rudiments are clearly visible. The transverse commissure between the cavities forms a mesenchymal condensation and later gives rise to the acrochordal cartilage, one of the earliest components of the chondrocranium. No structures homologous to the second and third pro-otic segments have been positively identified in birds, although Beer and Barrington (1934) noted that the cells forming the rudiment of the external rectus muscle (which arises from the third pro-otic somite in certain lower vertebrates) tend to arrange themselves radially around a small central cavity in the 28-somite duck embryo.

The formation of somites continues until approximately fifty pairs are present. This is the largest number observed in the goose, *Anser anser* (Gasser, 1877a), and the zebra parakeet, *Melopsittacus undulatus* (Abraham, 1901); as many as fifty-two have been counted in the chick (Williams, 1910), fifty-six in the ostrich, *Struthio camelus* (Boyden, 1922b). In the chick, the last pair of somites appears near the end of the fourth day of incubation. The rate at which mesodermal segmentation occurs, however, is greatly retarded by subnormal temperatures and only slightly accelerated by temperatures above the normal level of 37.5° C.; at 43.5° C., a delaying effect is again apparent. Figure 79 (see Chapter 3) shows the relationship between incubation temperature and the rate of formation of the first forty pairs of somites.

According to Holmdahl (1925b), all the somites posterior to those that will form the first or second lumbosacral vertebrae (the twenty-seventh or twenty-eighth pair, in the chick) are differentiated from the indifferent cell mass of the tail bud. When all the somites have appeared, a small caudal remnant of unsegmented mesoderm is left in the tip of the tail. Soon a regressive process begins in this mesodermal remnant and continues craniad, reducing the number of somites to the definitive total that is typical of the species (Holmdahl, 1925a).



*Early structural differentiation of the somites.* After its initial appearance, each somite differentiates into three portions, the dermatome, the myotome, and the sclerotome, which represent primordia of integumental, muscular, and skeletal tissues, respectively. At the time of its formation, every somite is slightly more advanced structurally than was the preceding somite when formed and somewhat larger; also, it passes more rapidly through the initial phases of differentiation. Nevertheless, the most anterior

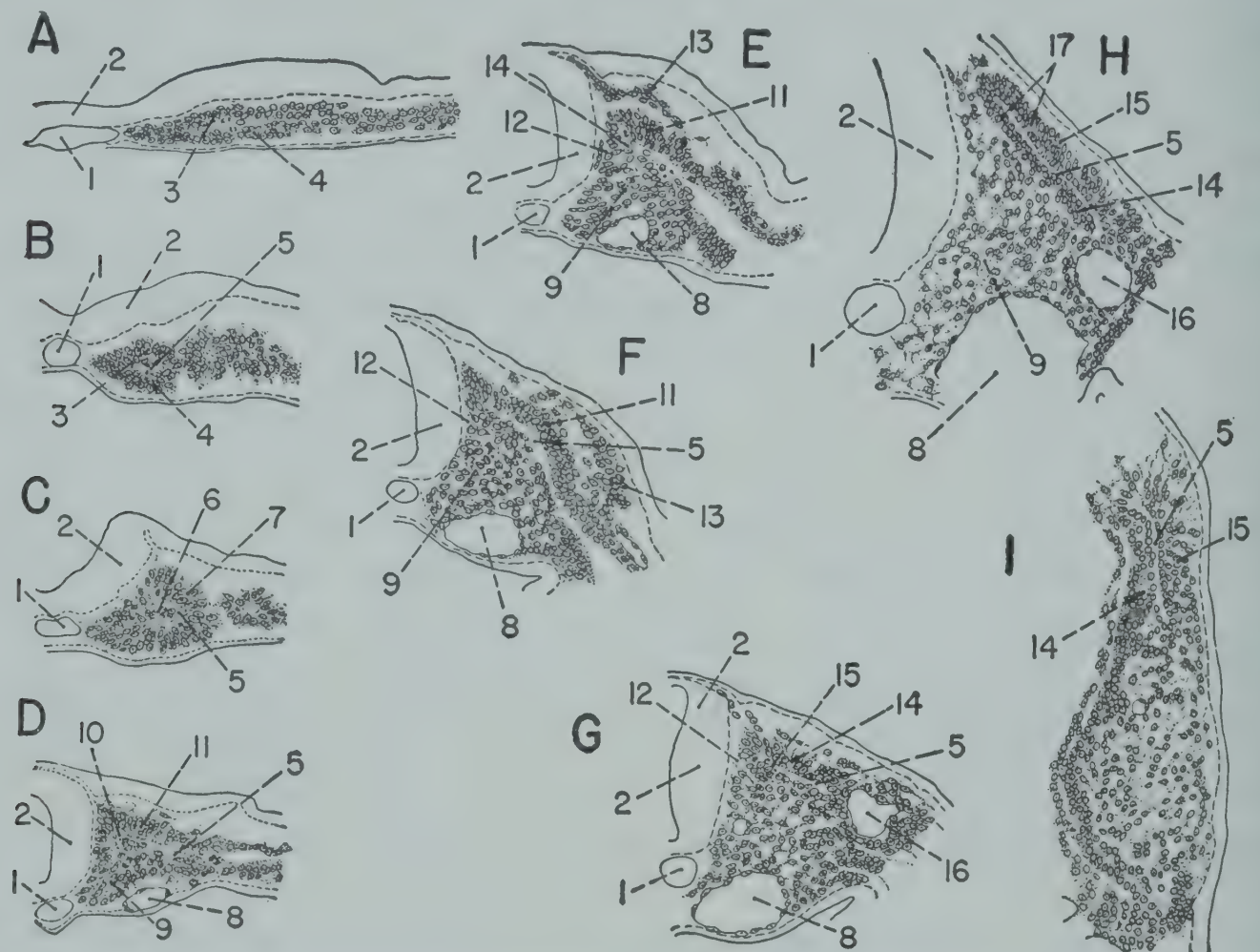


Fig. 344. Growth of the first somite from the 1-somite stage to the 39-somite stage, illustrating the increase in size characteristic of the anterior somites. (Redrawn with modifications after Williams, 1910.)

A, transverse section of one of the first pair of complete somites of a 1-somite embryo; B, of a 2-somite embryo; C, of a 5-somite embryo; D, of an 8-somite embryo; E, of an 11-somite embryo; F, of a 14-somite embryo; G, of a 17-somite embryo; H, of a 24-somite embryo; I, of a 39-somite embryo. All  $\times 90$ .

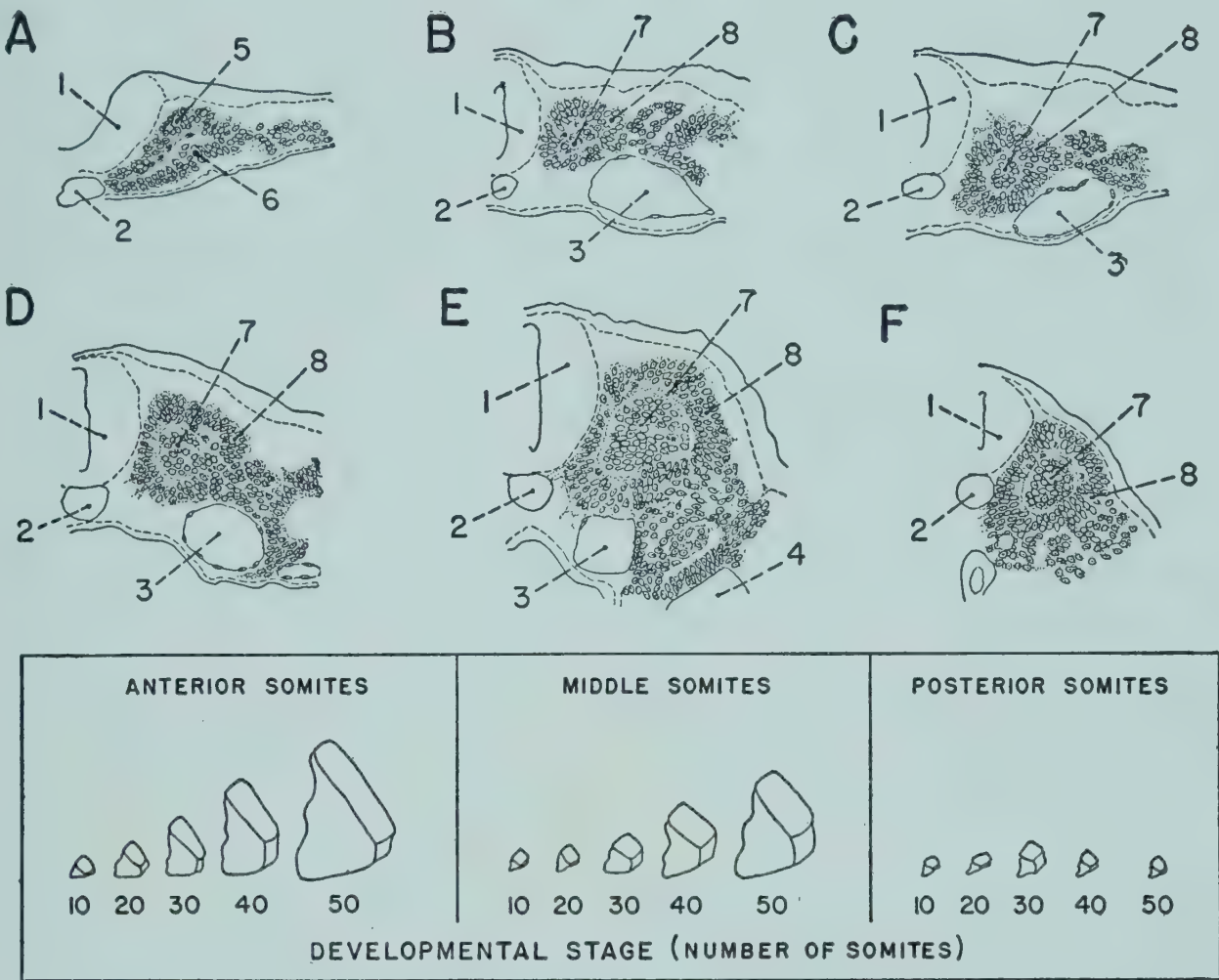
1, notochord; 2, neural tube; 3, upper layer of somitic cells; 4, lower layer of somitic cells; 5, cavity of somite; 6, core; 7, cortex; 8, aorta; 9, sclerotome; 10, upper myotomic groove; 11, dorsal lamella; 12, lower myotomic groove; 13, neural crest; 14, myotome; 15, dermatome; 16, anterior cardinal vein; 17, dermamyotome.

somites retain much of their early advantage, so that at any particular time they are somewhat more highly developed and of greater size than the more posterior somites. In addition, there are certain differences in the form of the somites in the various regions of the body.

Before any somites appear in the chick, the paraxial mesoderm becomes compact and at least four cells thick. As the first complete somite forms, its cells become fusiform and arranged radially in an upper and a lower layer,



each two cells thick (*Platt, 1889; Williams, 1910*). This somite is thus a flattened structure in the beginning (*Fig. 344-A and B*). By the 5-somite stage, the neural plate has become considerably elevated, and the first somite is now triangular in cross section, the medial half of its dorsal layer having followed the neural tube upward (*Fig. 344-C*). As the neural tube closes, the dorsomedial wall of the somite moves with it and, by the 8-somite stage, becomes the medial wall (*Fig. 344-D*), oriented vertically (*Williams, 1910*).



**Fig. 345.** A series of transverse sections through the last somite of chick embryos showing the increase in complexity of the somites at their origin, as they form in an anterior-posterior direction. (Redrawn with modifications after *Williams, 1910; Insert* after *Herrmann, Schneider, Neukom, and Moore, 1951*.)

A, from a 4-somite embryo; B, from a 9-somite embryo; C, from a 14-somite embryo; D, from a 24-somite embryo; E, from a 29-somite embryo; F, from a 43-somite embryo.

*Insert:* difference in growth rates of somites from anterior, middle, and posterior regions.

1, neural tube; 2, notochord; 3, aorta; 4, coelom; 5, upper layer of somitic cells; 6, lower layer of somitic cells; 7, core; 8, cortex.

In contrast, the fourth somite is already somewhat triangular in cross section when it appears. More posterior somites, when transected, prove to have from four to six sides of unequal length at the time of their formation (*Fig. 345*).

At the 5- or 6-somite stage, a few cells appear between the upper and lower layers of the first somite (cf. *Fig. 344-C*). These cells constitute the core. Beginning with the ninth segment (*Williams, 1910*), all newly



formed somites consist of a central core surrounded by a cortex of radially arranged cells (*Ilis, 1868*), as Fig. 345 shows. A relative and absolute increase in the size of the core (*Williams, 1910*) accounts largely for the initially greater dimensions of each succeeding somite down to the thirtieth or thirty-fifth (*Herrmann, Schneider, Neukom, and Moore, 1951*).

Between the 6- and the 12-somite stages, the core of each of the first three or four somites becomes separated from the dorsal wall by a flattened cavity, which is continuous laterally with the coelom. This communication persists no later than the 20-somite stage and is obliterated by the development of the anterior cardinal vein. There is no continuity between the somitic and coelomic cavities in the more posterior somites (*Dexter, 1891; Williams, 1910*).

By the 8- or 9-somite stage, the cells of the dorsal wall of the first somite have become arranged as a stratified columnar epithelium lying directly above the cavity of the somite. Cells proliferated from the edges of this dorsal lamella will be contributed to the myotome and the sclerotome. The earliest indication of myotome formation can now be seen at the dorso-medial edge of the somite, where the cellular arrangement is such that the dorsal lamella appears to be continued ventrally for a short distance into the thin upper end of the medial wall (Fig. 344-D). The core and floor of the somite and the lower part of the medial and anterior walls are beginning to be transformed into mesenchyme and to form a mass of tissue that can be regarded as the primary sclerotome. The ninth somite reaches this stage by the time the twelfth somite appears (*Williams, 1910*).

In the embryo of 11 or 12 somites, the myotome of the first somite is recognizable as a short and narrow zone of oval cells, which is continuous with the dorsal lamella around the medial end of the cavity of the somite. A groove on the medial surface of the somite demarcates the myotome ventromedially. The sclerotome has been enlarged by the addition of more cells from the anterior and medial walls of the somite (cf. Fig. 344-E). By the 14- or 15-somite stage, the myotome starts to grow laterally beneath the dorsal lamella, or dermatome, and its cells begin to be aligned longitudinally rather than transversely (cf. Fig. 344-F). The medial end of the somitic cavity is enclosed between the dermatome and the myotome. The groove bounding the myotome ventrally has extended from the medial surface of the somite on to the anterior and posterior surfaces as well. The ninth somite attains an equivalent degree of development between the 17- and 20-somite stages, but the twenty-fourth somite differentiates so rapidly that it is even more advanced by the stage of only 27 somites (*Williams, 1910*) (cf. Fig. 344-G, H, and I).

In the first somite of the 18-somite embryo, the myotome, bounded ventrally by a narrow cleft, underlies the medial half of the dermatome. The sclerotome is much larger; it borders the ventrolateral half of the neural



tube and is extending ventromedially toward the notochord (Williams, 1910). In the duck (*Anas platyrhynchos*), the second metotic somite has a complete dermatome and myotome at this stage but is continuous anteriorly with the unsegmented mesoderm (Beer and Barrington, 1934).

In the chick embryo, differentiation into a sclerotome, dermatome, and myotome is complete in the first, ninth, and twenty-fourth somites at the stages of 24, 30, and 33 somites, respectively (Williams, 1910). The myo-

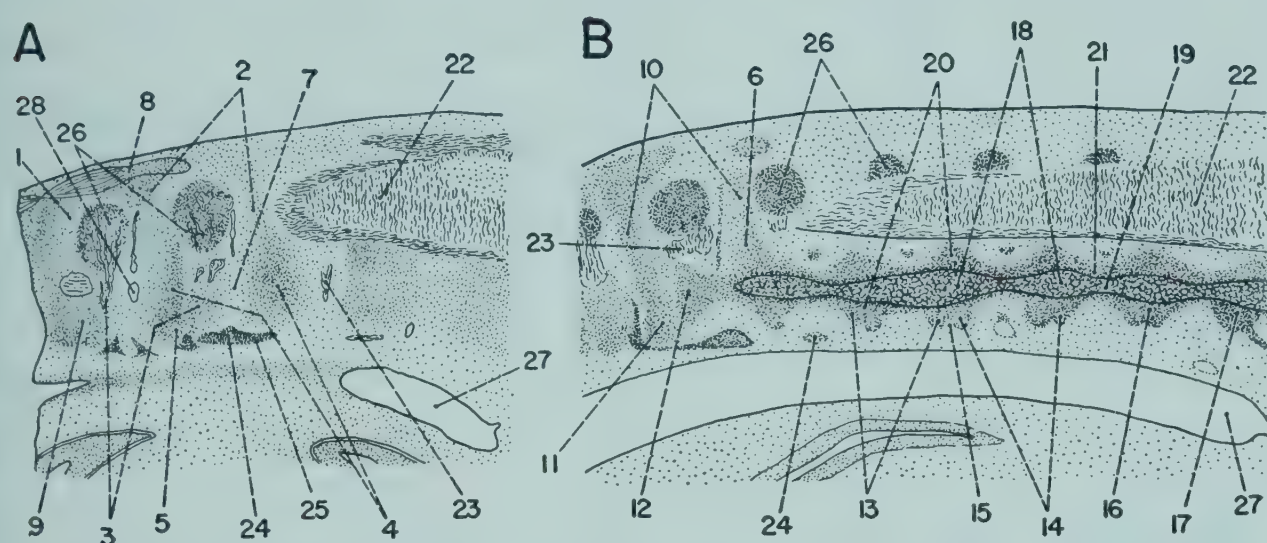


Fig. 346. Differentiation of the sclerotomal plate into arcualia, as demonstrated in sagittal sections of the embryo gull, *Larus canus*. (Redrawn with modifications after Piiper, 1928.)

The cervical region of the gull embryo, showing an early stage in the establishment of an intermyotomal segmentation plan. The anterior end of the section is to the right. A, lateral sagittal section from a 3-day embryo; B, the same, but a more mesial section than in A, from a 4-day embryo. Both  $\times 35$ .

1, neural portion of the cranial sclerotomite; 2, neural portion of the caudal sclerotomite; 3, chordal portion of the cranial sclerotomite; 4, chordal portion of the caudal sclerotomite; 5, bridge of cells connecting in subchordal region the cranial and caudal sclerotomites of a sclerotome; 6, caudal sclerotomite; 7, intersclerotic fissure; 8, intermyotomic septum; 9, intrasclerotic fissure; 10, basidorsal; 11, basiventral; 12, interven-tral; 13, ventralmost portion of basiventrals; 14, ventralmost portion of interven-trals; 15, transverse groove separating the subchordal portion of the basiventrals from the corresponding portion of the interven-trals; 16, axis intercentrum; 17, atlas intercentrum; 18, dilated portion of notochord; 19, constricted portion of notochord; 20, perichordal ring; 21, perichordal tube; 22, neural cord; 23, spinal nerve; 24, sympathetic ganglion; 25, sympathetic cord; 26, spinal ganglion; 27, radix aortae; 28, intersclerotic blood vessel.

tome is a somewhat thinner lamella than the dermatome and extends to its lateral edge; together, these two plates of tissue, enclosing the original cavity of the somite between them, are inclined downward laterally at an angle of  $45^\circ$ . The myotome is separated from the sclerotome by a narrow fissure, or myocoele, Fig. 346-A and B (Piiper, 1928). The sclerotome extends farther dorsad than before; also, it is united beneath the notochord with the sclerotome of the opposite side (Williams, 1910). The lateral portion of the sclerotome has become denser than the medial portion and



is beginning to be divided into a cranial and a caudal half, or sclerotomite, by a narrow zone where the cells are sparser than elsewhere (Williams, 1942). This zone is the incipient intrasclerotomic fissure, or sclerocoel, first seen by Ebner (1888). The caudal sclerotomite is somewhat denser than the cranial sclerotomite (Piiper, 1928). This stage of development is found in the cervical region of the 2-day gull (*Larus canus*) embryo, Fig. 347-A<sub>1</sub> and A<sub>2</sub> (Piiper, 1928), which is comparable with the 55-hour chick

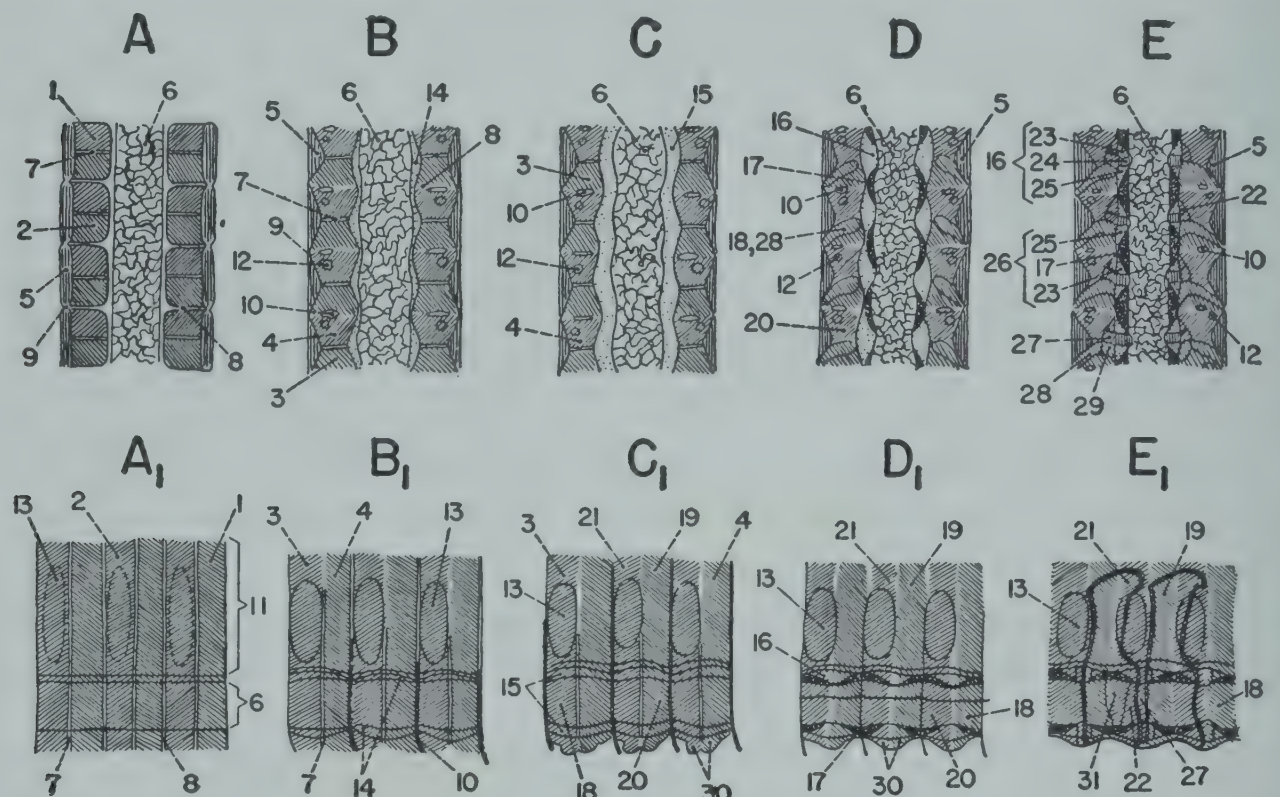


Fig. 347. Diagrams of successive stages in the development of the cervical vertebrae in the gull (*Larus canus*) showing the relationship of the vertebral parts to the chorda and perichordal tube. (Redrawn with modifications after Piiper, 1928.)

A, A<sub>1</sub>, frontal and sagittal sections of a cervical somite at the time when the dermatomyotome and sclerotome have differentiated, and the sclerotome is divided into cranial and caudal sclerotomites (2-day embryo); B, B<sub>1</sub>, frontal and sagittal sections of vertebra when the notochord begins to take on a moniliform appearance (3-day embryo); C, C<sub>1</sub>, frontal and sagittal sections of the stage when the intrasclerotomic fissure is almost obliterated and the perichordal tube is completely formed (4-day embryo); D, D<sub>1</sub>, frontal and sagittal sections of the stage when the perichordal tube becomes differentiated into two alternating structures, intravertebrally disposed vertebral rings and the intervertebrally located interstitial bodies (5-day embryo); E, E<sub>1</sub>, frontal and sagittal sections of the vertebra when the middle portion of the interstitial body differentiates into the primary intervertebral body. The vertebral rings combine with the prospondylous and opisthospondylous zones to form the primary vertebral body. Ribs begin to develop; chondrification of the vertebral parts begins (6-day embryo).

1, primary cranial sclerotomite; 2, primary caudal sclerotomite; 3, secondary cranial sclerotomite; 4, secondary caudal sclerotomite; 5, myotome; 6, chorda; 7, intrasclerotomic fissure; 8, intersclerotomic fissure; 9, intermyotomic septum; 10, intersclerotomic blood vessel; 11, neural cord; 12, spinal nerve; 13, spinal ganglion; 14, perichordal ring; 15, perichordal tube; 16, interstitial body; 17, vertebral ring; 18, basiventral; 19, basidorsal; 20, interventral; 21, dorsal-interdorsal; 22, anterior portion of interventral; 23, opisthospondylous zone; 24, primary intervertebral body; 25, prospondylous zone; 26, primary vertebral body; 27, posterior portion of interventral-intervertebral ligament; 28, rib; 29, parapophysis; 30, intercentrum; 31, interdorsal.



(Williams, 1942). The next step is the growth of sclerotomic mesenchyme between the neural tube and the notochord, so that the latter is surrounded by cells.

The somites grow, of course, while differentiating. Up to the stage of 50 segments, dimensional increase occurs in the most anterior somites, as would be expected. In the chick, the width and depth of these somites are about 160 and 130 microns, respectively, at the 6- to 10-somite stage and increase to about 1250 and 1390 microns, respectively, by the 50-somite stage; the length simultaneously increases only from 145 microns to 275 microns. The twentieth somite, which is 180 microns wide, 195 microns deep, and 200 microns long shortly after its appearance, grows to be 815 microns wide, 1020 microns deep, and 365 microns long while the remainder of the somites are forming. In the anterior somites, the growth rate is highest at the 15- to 25-somite stage, but the relatively greatest increase in the size of the twentieth somite occurs at stages between 25 and 35 somites. The amounts of nitrogen, ribose nucleic acid, and desoxyribose nucleic acid in the somites rise most rapidly between the 20- and 25-somite stages and between the 45- and 50-somite stages (Herrmann, Schneider, Neukom, and Moore, 1951).

### *The Vertebral Column*

In the vertebral column of birds, it is possible to distinguish cervical, thoracic, lumbar, sacral, and caudal regions. The number of vertebrae in each region varies among the different species, as does the total number of vertebrae, which may range from forty in finches, *Fringillidae*, to fifty-nine or sixty-one in such ratite birds as the cassowary (*Casuarius* sp.) and the emu (*Dromaeus novae-hollandiae*). There is a tendency for the thoracic vertebrae to be ankylosed, and several caudal (and usually thoracic) vertebrae have coalesced with the lumbar and sacral vertebrae to form the so-called synsacrum, which in turn is united with the pelvic girdle. In all but a few birds, several of the most posterior caudal vertebrae are fused together and comprise the pygostyle, the structure supporting the tail feathers; but the anterior caudal vertebrae remain free.

A typical vertebra is composed of a body, or centrum, ventral to the spinal cord, and a neural arch dorsal to it. Each centrum possesses an anterior and a posterior articulating surface. The neural arch is provided with a median dorsal spinous process, a transverse process or diapophysis on either side, and paired articulating processes or zygapophyses anteriorly and posteriorly. The anterior zygapophyses (prezygapophyses) of each vertebra articulate with the posterior zygapophyses (postzygapophyses) of the succeeding vertebra. The individual free vertebrae vary somewhat in structure in the different regions. For example, in the thoracic region, each diapophysis articulates with the tubercle of a rib, and there is a



parapophysis on each lateral surface of the centrum to articulate with the head of a rib. In the cervical region, the ribs are rudimentary, and their heads and tubercles are fused to the vertebrae; the ribs thus form costal processes pierced by a foramen, which is occupied by the vertebral artery. The first two cervical vertebrae, the atlas and the axis, are modified to permit the bird's head to turn.

The vertebrae form between rather than within the embryonic segments; as Remak (1855) was the first to note, an individual vertebra represents a recombination of the caudal and cranial halves of two adjacent segments. The first cervical vertebra or atlas is derived from halves of the fifth and sixth permanent somites (the tenth and eleventh of the entire series); the more anterior segments are incorporated into the head.

The first sclerotomic derivatives involved in the development of the vertebrae and the intervertebral ligaments are a layer of perichordal tissue and several more peripheral differentiations, the primordia of cartilages known as arcualia. The cartilaginous vertebral centra are the result of the fusion of certain arcualia with annular perichordal structures; the neural arches are formed of arcualia alone. Neural arches do not develop when the neural tube has been removed at the 15-somite stage (*Strudel, 1953*).

Ossification of the vertebrae begins late in incubation and is not complete at hatching. There are fewer independent ossification centers than chondrification centers.

The development of the vertebral column begins anteriorly and proceeds caudad. At any particular time, however, regional differences are not extremely marked, although the cervical and thoracic vertebrae are more advanced than the sacral and caudal elements.

**The Cervical Vertebrae.** The development of the entire vertebral column is the result of the same basic processes that are involved in the formation of the cervical vertebrae, hence only the cervical region need be discussed in detail.

The transition from the end stages of somite differentiation to the initial stages of vertebral development is not sharp. For convenience, it can be arbitrarily identified as falling within the period when the sclerotome divides into cranial and caudal sclerotomites and the sclerotomic mesenchyme grows completely around the notochord to form the cellular component of the perichordal sheath.

The production of mesenchymal cells by the sclerotome is most intense in the region of the intrasclerotomic fissure. Consequently, a thickened ring is soon formed medially around the notochord, opposite each fissure. The cells of the perichordal rings are fusiform, surrounded by intercellular substance, and arranged concentrically around the chorda, which is slightly dilated at the level of each ring. The sclerotomites consist of isodiametric polygonal cells with a small amount of intercellular substance. The cells



of the upper or neural portion of each caudal sclerotomite radiate from the notochord; the upper part of the cranial sclerotomite is occupied largely by the spinal ganglion. This is the developmental stage seen in the 3-day embryo of the gull, *Larus canus* (Piiper, 1928), and in the 85-hour chick embryo (Williams, 1942).

In the 4-day gull (*Larus canus*) or 4.5-day chick embryo, the perichordal rings have fused to form a continuous tube surrounding the moniliform notochord (cf. Fig. 347-B and B<sub>1</sub>). The intersclerotomic (that is, intersomitic) clefts are almost completely obliterated, and the intrasclerotomic fissures are losing their distinctness medially and laterally (Fig. 347-C and C<sub>1</sub>). Precursors of the arcualia have now appeared. Ventral and ventrolateral to the notochord, a condensation of concentrically arranged cells in the anterior part of each caudal sclerotomite, just behind the disappearing intrasclerotomic fissure, represents a subchordally fused pair of prechondral basiventral arcualia; immediately anterior to the basiventrals, in the posterior part of each cranial sclerotomite, a similar formation of less advanced and not definitely oriented cells represents a subchordally fused pair of interventral arcualia. Together, the adjacent subchordal portions of a pair of basiventrals and a pair of interventrals form an intercentrum (Piiper, 1928) which occupies an intervertebral position and is equivalent to the "subnotochordal bar of Froriep (1883)"; however, it is usually interpreted as representing only basiventrals. The neural portions of the caudal sclerotomites represent the basidorsal arcualia, membranous dorsally but consisting of incipient prechondral tissue basally, where they abut against the dorsolateral surfaces of the perichordal tube. Cell condensations in the connective tissue of the upper portions of the cranial sclerotomites, above the spinal ganglia, represent the dorsal parts of the interdorsal arcualia, that is, the dorsal-interdorsals.

The next stage is exemplified by the 5-day chick (Froriep, 1883), or the 8-day ostrich (*Struthio camelus*) embryo (Piiper, 1928). Concentric rings of fusiform cells with abundant intercellular substance have appeared in the thinner portions of the perichordal tube, between the thicker portions, that is, at intersclerotomic intervals. These rings represent the internal and middle (waist) portions of the vertebral centra (Sonies, 1907), hence may be called vertebral rings (Fig. 347-D and D<sub>1</sub>). According to Piiper (1928), the anterior half of each vertebral ring is formed by the caudal end of a perichordal ring, and the posterior half is formed by the cranial end of the next more posterior perichordal ring; according to Froriep (1883), the rudimentary vertebral bodies are new differentiations, and the perichordal rings represent the primordia of the intervertebral ligaments. Piiper (1928) stated that only the thick middle zones of the perichordal rings remain between the vertebral rings as interstitial bodies, covered ventrally by the intercentra. The interstitial bodies consist of irregularly



oriented cells without stainable intercellular substance. The notochord is now constricted opposite the interstitial bodies (*Froriep*, 1883), where it was formerly dilated.

In the 5-day gull (*Larus canus*) embryo (*Piiper*, 1928), each vertebral ring, now cartilaginous, has become a primary vertebral body or centrum through the addition of a prechondral zone at its anterior and its posterior end. The ends have thus increased in diameter relative to the midportion or waist. In Piiper's (1928) scheme, the anterior or prospondylous zone is the former caudal end of an interstitial body, and the posterior or opisthospodylous zone is the former cranial end of an interstitial body (cf. Fig. 347-E and  $E_1$ ). The middle segment of each interstitial body remains as a primary intervertebral body, consisting of transversely disposed cells with faint traces of intercellular substance. The intrasclerotomic fissure has completely disappeared; it is clear, therefore, that it corresponds to the intervertebral fissure only in position (*Ebner*, 1888). Piiper (1928) found that the intercentrals are now divided into an anterior and a posterior part. The subchordal, unpaired portion of the posterior division (that is, the intervertebral segment of the intercentrum), still consisting of connective tissue, has undergone great reduction. The dorsal ends of successive basidorsals are connected by the dorsal-interdorsals, now prechondral; in parasagittal sections, these connections appear as a series of arches (Fig. 348). As in the 5-day chick (*Froriep*, 1883) the formation of the vertebral artery and the collateral sympathetic cord on each side has separated the distal portion of the caudal sclerotomite from the basiventral; each rib (costal process) arises as an independent differentiation in this part of the caudal sclerotomite (*Sonies*, 1907; *Piiper*, 1928; *Williams*, 1942). In the gull (*Larus canus*), the rib is now represented by early prechondral tissue in the form of a forked rudiment whose dorsal limb is connected with the ventrolateral portion of the basidorsal (the future diapophysis) and whose ventral limb is continuous with the dorsolateral portion of the basiventral (the future parapophysis). A somewhat different account of the origin of the duck's (*Anas platyrhynchos*) ribs was given by *Sonies* (1907), who stated that the dorsal ends of the rib primordia gradually approach the vertebrae and then bifurcate.

The transformation of the primary vertebral body into the secondary vertebral body through fusion with arcualia is apparent in the 5.5-day chick embryo (*Williams*, 1942) and the 10- or 12-day ostrich (*Struthio camelus*) embryo (*Piiper*, 1928). The paired interdorsals have now differentiated from tissue covering the vertebral ring dorsolaterally; from the posterior edges of the basal parts of the basidorsals they extend backward and downward to the intercentrals, and form most of the dorsolateral aspect of the secondary vertebral body. The prechondral basiventrals have spread to cover the ventral and ventrolateral surfaces of the opistho-



spondylous zone and are continuous dorsally with the basidorsals. The anterior parts of the interventrals, also precartilaginous, have fused with the ventrolateral surfaces of the opisthospondylous zone; the product of this fusion probably corresponds to the rudimentary vertebral bodies that Froriep (1883) described (Piiper, 1928). The posterior, lateral parts of the interventrals constitute the outer layer of the secondary intervertebral body. In the chick, the unpaired interventral portion of the intercentrum has disappeared at this stage. The vertebral body and neural arches are

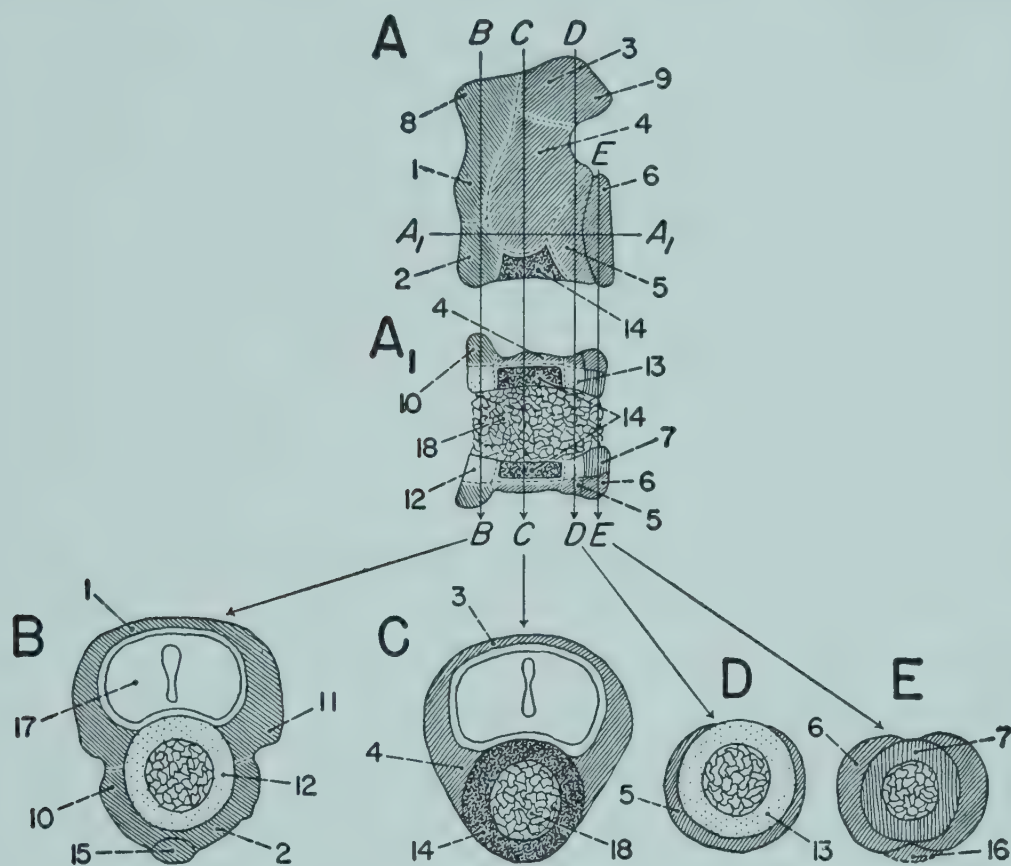


Fig. 348. Diagram illustrating the composition of a cartilaginous bird vertebra. (Redrawn with modifications after Piiper, 1928.)

A, lateral view of vertebra and intervertebral body; A<sub>1</sub>, frontal section of vertebra; B, C, D, E, transverse sections through the vertebra at position indicated by vertical lines.

1-6, arcualia: 1, basidorsal, 2, basiventral, 3, dorsal-interdorsal, 4, interdorsal, 5, anterior portion of interventral, 6, posterior portion of interventral; 7, primary intervertebral body (suspensory ligament); 8, anterior zygapophysis; 9, posterior zygapophysis; 10, parapophysis of basiventral; 11, diapophysis; 12, prospondylous zone; 13, opisthospondylous zone; 14, vertebral ring; 15, intercentrum; 16, anterior atrophied portion of intercentrum; 17, neural cord; 18, chorda.

covered externally by the connective tissue of the perichondrium (Schwarck, 1873).

In the 6-day gull (*Larus canus*) embryo (Piiper, 1928), both interventral and basiventral portions of all cervical (except atlas and axis) intercentra have atrophied, as they have in the 7- or 7.5-day duck (*Anas platyrhynchos*) embryo (Sonies, 1907). In the chick embryo the basiventral components of the last three or four cervical intercentra persist and they chondrify during the sixth day (Williams, 1942), simultaneously with the secondary



vertebral bodies and the lateral parts of the neural arches; dorsally, the neural arches are completed by the fibrous membrana reuniens (*Froriep*, 1883). The pro- and opisthospondylous zones have grown thicker, so that the vertebral body appears considerably swollen at each end in the 6-day gull. The parapophyses are prechondral and the capitula of the ribs are partly chondrified. The external (interventral) portion of the secondary intervertebral body is no longer prechondral but consists of a connective tissue. The craniocaudal extent of the intervertebral body is less than before (*Piiper*, 1928).

In the gull (*Larus canus*) incubated 8 days, the posterior ends of the fully chondrified dorsal-interdorsals (that is, the rudimentary postzygapophyses) are connected with the anterior faces of the next more caudal basidorsals (that is, the rudimentary prezygapophyses) by connective tissue only. Consequently, the dorsal-interdorsals now appear as caudal processes of the dorsal ends of the basidorsals. Through the growth of the interdorsals, the neural arch has increased in craniocaudal extent. The membrana reuniens is chondrifying, as it is in the 8-day chick (*Froriep*, 1883) and 10-day duck (*Anas platyrhynchos*) embryo (*Sonies*, 1907). In the gull (*Larus canus*) embryo incubated 10 days (*Piiper*, 1928), the dorsal growth of the interdorsals and their fusion to the ventral surfaces of the anterior portions of the dorsal-interdorsals have resulted in the formation of the definitive neural arch, which consists of basidorsals anteriorly and of interdorsals and dorsal-interdorsals posteriorly. Like the vertebral body, the neural arch is the product of two different sclerotomes (*Schauinsland*, 1906; *Piiper*, 1928). In the 10-day chick embryo, a joint cavity with articular surfaces has formed between the pre- and postzygapophyses (*Williams*, 1942). The ribs, although chondrified on the eighth day in the chick (*Froriep*, 1883) and the gull (*Piiper*, 1928), are separated from the parapophyses and diapophyses by small zones of prechondral tissue, but these zones chondrify on the ninth or tenth day in the cervical vertebrae of the gull (*Piiper*, 1928). On the gull's eighth day (*Piiper*, 1928), the notochord forms a secondary dilatation and constriction near each end of the vertebral body, opposite the thickened pro- and opisthospondylous zones. During the next 2 days of incubation, intense growth at the waist of the vertebral body, in the medial region corresponding to the vertebral ring, causes the notochord to appear constricted within the centrum and dilated intervertebrally, as it was in the beginning. This condition is seen on the tenth or twelfth day in the duck, *Anas platyrhynchos* (*Sonies*, 1907), and on the eighth day in the chick; but, it has been reported that later in incubation, the maximum constriction of the chick's notochord is once more opposite the intervertebral body (*Gegenbaur*, 1872; *Froriep*, 1883), where, on the twelfth or fourteenth day, the regressing notochord is triangular in section (*Schwarck*, 1873).



The cervical vertebrae are the first in which ossification begins, but, like all the vertebrae, they are still partly cartilaginous at the end of incubation. The ossification centers do not coincide exactly with the chondrification centers (*Parker, 1892a*). Although ossification always starts in the vertebral body, its course varies slightly among different species of birds. In the chick, it is initiated on the twelfth or thirteenth day of incubation (*Gegenbaur, 1872; Schwarck, 1873*). As in the pigeon (*Columba livia*) the first center of ossification (perichondral) takes the form of a flat plate on the dorsal surface of the vertebral body (*Schinz and Zangerl, 1937*). At the level of the waist of the centrum, there is a medial center of ossification

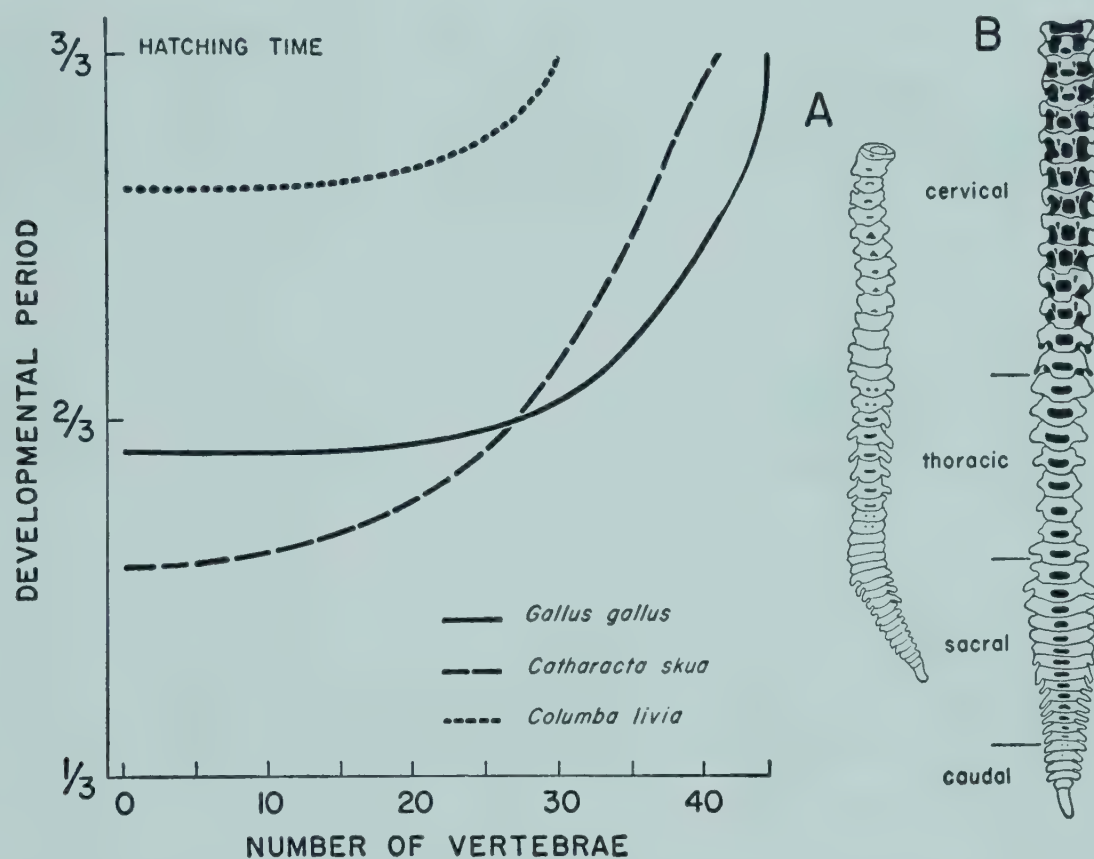


Fig. 349. A graphic comparison of the beginning of ossification in the vertebral column in three avian species: pigeon, *Columba livia*, chick, *Gallus gallus*, and skua gull, *Catharacta skua*. (Redrawn with modifications after Maillard, 1948.)

A and B are diagrammatic representations of the ossification centers in the skua gull, 17- and 24-day embryo, respectively. Both natural size.

(endochondral) in the vicinity of large, markedly granular cells situated just lateral to the cellular sheath of the notochord (*Schwarck, 1873*). A third center (perichondral) then appears on the ventral surface of the vertebral body; it is flat like the dorsal center (*Schinz and Zangerl, 1937*). Ossification spreads peripherally from the medial center and is most rapid near the ends of the centrum, opposite the constrictions of the notochord (*Schwarck, 1873*). In the great skua (*Catharacta skua*) whose incubation period is about 30 days, the dorsal center and a pair of ventral centers appear on the sixteenth day of incubation (Fig. 349), and the ventral centers fuse together during the next 2 or 3 days. The medial center does not appear until the twenty-fourth day, when it unites the enlarged dorsal



and ventral centers (Maillard, 1948); and it is similarly the last of the three to appear in the crested grebe, *Podiceps cristatus* (Schinz and Zangerl, 1937). In the great skua, ossification of the cervical neural arches and costal processes begins on the twenty-third and twenty-fourth days, respectively. At the end of incubation, the three centers in each cervical vertebral body have fused into one, and both the body and the neural arch are largely but not completely ossified; the spinous process does not ossify until 2 to 4 days after hatching (Maillard, 1948). In the newly

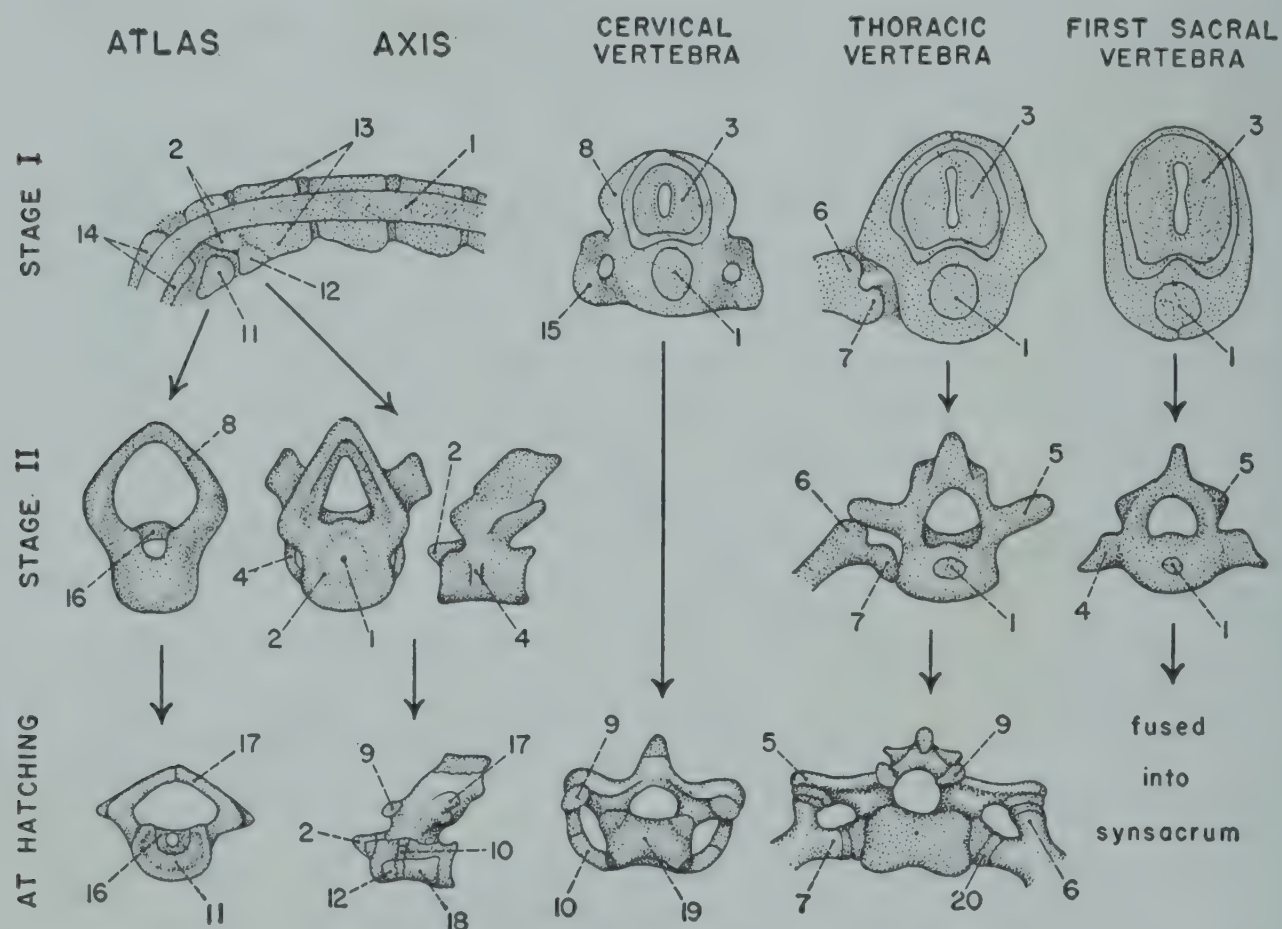


Fig. 350. Successive stages in the chondrification and ossification of vertebrae from different regions of the spinal column in the kiwi embryos, *Apteryx australis*, *Apteryx oweni*. (Redrawn with rearrangement after T.J. Parker, 1892a.)

Stage I,  $\times 10$ ; Stage II,  $\times 8$ ; at hatching,  $\times 2$ .

1, notochord; 2, odontoid; 3, myelon; 4, pleuroid; 5, dispophysis; 6, tubercle of rib; 7, head of rib; 8, neuroid; 9, anterior zygapophysis; 10, pleurosteite; 11, post-occipital intercentrum; 12, post-atlantal intercentrum; 13, centrochondrite; 14, occipital condyle; 15, pleurochondrite; 16, ligament; 17, neurosteite; 18, centrosteite; 19, neurocentral suture; 20, parapophysis.

hatched kiwi, *Apteryx australis* (Fig. 350), ossification is spreading from the neural arch into the dorsolateral portions of the centrum and has begun in the ventral part of each costal process (Parker, 1892a).

In the chick embryo, the intervertebral bodies start to form the intervertebral ligaments between the fifth and eighth days (Froriep, 1883). The small, densely set medial elements lose their concentric orientation and spread apart; these cells will comprise the suspensory ligament, which is a band stretching across the articular cavity between the ends of two suc-



cessive vertebrae (Jager, 1858; Schwarck, 1873). The central location of the suspensory ligament indicates that it is probably a derivative of the perichordal ring. The more external intervertebral ligament or meniscus develops from the outer part of the intervertebral body, therefore probably from the intercentrals (Piiper, 1928). It is seen in the 14-day chick embryo in the form of two wedge-shaped lateral halves whose apexes are directed internally. It is continuous with the suspensory ligament. The joint cavity forms from the periphery inward as the result of the resorption of cells. The perichondrium is unchanged by joint formation and stretches from one vertebra to the next, remaining in continuity with the intervertebral ligament (Schwarck, 1873).

*The atlas and the axis.* The first cervical vertebra or atlas, which articulates with the occipital condyle of the skull, is essentially a ringlike structure; its centrum is detached from its neural arch and is fused with the centrum of the second cervical vertebra, or axis, to form the odontoid process of the latter. The intercentrum of the atlas is retained in place of the centrum, and the intercentrum of the axis also persists, fused with the atlas and axis bodies (Froriep, 1883). The atlas neural arch is reduced in craniocaudal extent as compared with other cervical neural arches.

The atlas and axis of the 3-day gull (*Larus canus*) embryo (Piiper, 1928) are distinguishable from the remaining cervical sclerotomes only by the absence or rudimentary condition of the spinal ganglia. In the 4-day embryo, however, it can be seen that the atlas dorsal-interdorsal formations are much reduced, and that the axis and atlas intercentra are relatively large. Both interdorsals and dorsal-interdorsals are entirely lacking in the atlas of the 5-day embryo, but the basidorsals, which will alone form the dorsal half of the definitive neural arch, are well developed. The atlas intercentrum, anterior to the atlas centrum, is now about twice as large as any other cervical intercentrum. The prospondylous and opisthospodylous zones are nearly absent from the atlas centrum. The intercentrum of the axis is attached to the anteroventral face of the axis centrum. Prechondral rudiments of costal processes are forming on both the atlas and the axis.

The definitive structure of the atlas and axis is indicated in the 8-day chick (Froriep, 1883), the 9- or 10-day duck, *Anas platyrhynchos* (Sonies, 1907), and the 6-day gull, *Larus canus*, embryo (Piiper, 1928). The centra of the two vertebrae are beginning to fuse with each other and with the axis intercentrum. The intercentrum of the atlas is continuous laterally with the atlas basiventrals, and the upper ends of the basiventrals are fused with the basal portions of the basidorsals; but all these structures are completely separate from the atlas centrum, the primary vertebral body (Piiper, 1928).

In the 8-day chick (Froriep, 1883) or gull, *Larus canus* (Piiper, 1928),



the axis and the atlas ribs are still prechondral, whereas all other cervical ribs have chondrified. The atlas and axis neural arches are closed dorsally (*Froriep*, 1883). In the kiwi (*Apteryx oweni*) chondrification of the atlas neural arch seems to be relatively somewhat later; a small middorsal segment of prechondral tissue remains at a time corresponding to the chick's tenth day. The axis now consists of solid cartilage (cf. Fig. 350). The position of the greatly reduced notochord is indicated by a small pit on the anterior surface of the odontoid process (*Parker*, 1892a).

In the kiwi (*Apteryx oweni*), ossification is apparent in the neural arch of the atlas and in the odontoid process and body of the axis before it begins in the intercentra. At hatching, there are three centers of ossification in the atlas: one in the "body," or intercentrum, and one on each side of the neural arch (Fig. 350). In the axis, there are seven centers of ossification: one each in the body, the intercentrum, and the odontoid process, two in the neural arch, and one in each costal process (Fig. 350). The union of the separate elements of the atlas and axis is not complete until some time after hatching (*Parker*, 1892a).

**The Thoracic Vertebrae.** Except for the detachment and better expression of their ribs, the thoracic vertebrae develop in essentially the same manner as the cervical vertebrae and almost at the same time (cf. Fig. 350). In fact, in the great skua (*Catharacta skua*) ossification begins in the thoracic vertebrae 1 or 2 days before it is seen in several of the most posterior cervical vertebrae (cf. Fig. 349). In this bird, it is said the paired ventral ossification centers appear in the bodies of the thoracic vertebra before the dorsal centers (*Maillard*, 1948).

In many birds, a few of the most posterior thoracic vertebrae are incorporated into the synsacrum.

**The synsacrum.** The synsacrum, which provides support for the long ilia, is composed of a variable number of fused vertebrae, most commonly from eleven to seventeen. The synsacrum usually consists of vertebrae of the lumbar, sacral, and anterior caudal regions but may also contain some of the most posterior thoracic elements, as it does in kiwis (*Apteryx australis*, *Apteryx oweni*), for example (*Parker*, 1892a). Costal processes may be present on various synsacral vertebrae (except those of the lumbar region) and may be prominent on the true sacral vertebrae, as they are in the chick (*Schinz and Zangerl*, 1937) and the kiwi, *Apteryx oweni* (*Parker*, 1892a).

The synsacral region can be differentiated in the 4-day gull (*Larus canus*) embryo. At lumbar, sacral, and anterior caudal levels, the cranial sclerotomites are fused with the caudal sclerotomites subchordally, and thus interventral and basiventral divisions of the intercentra are not distinguishable. Instead, the intercentra are divided into lateral halves by a sagittal groove, which is occupied by the dorsal part of the dorsal aorta.



Although the synsacral intercentra, like those at more cranial levels, begin to chondrify on the sixth day, they have almost completely atrophied by the end of the eighth day (*Piiper, 1928*). At a period equivalent to the latter stage in the chick, the synsacral vertebrae of the kiwi (*Apteryx oweni*) are completely chondrified (Fig. 350—Stage I) except for the costal processes, which are apparent in kiwi embryos (*Apteryx australis*, *Apteryx oweni*) corresponding to 11- or 12-day chick embryos (*Parker, 1892a*).

The chondrification of the intervertebral bodies, which begins on the gull's (*Larus canus*) tenth day, leads to the fusion of the synsacral centra. Chondrification starts externally, in the intervertebral ligament, and proceeds toward the interior, eventually affecting the suspensory ligament also (*Piiper, 1928*). The intervertebral bodies have disappeared in the synsacral region of the 14-day chick embryo (*Schwarck, 1873*). In the 10-day gull (*Larus canus*), the successive synsacral neural arches have fused dorsally but not ventrally (*Piiper, 1928*).

In the chick, pigeon, *Columba livia* (*Schinz and Zangerl, 1937*), and great skua, *Catharacta skua* (*Maillard, 1948*), a few of the most anterior synsacral vertebrae begin to ossify at about the same time as the thoracic vertebrae, but ossification advances through the remainder of the synsacral region at a slower rate. Thus ossification of the last synsacral vertebra starts on the seventeenth day in the chick, on the twenty-fourth day in the skua, and on the last day of incubation in the pigeon (Fig. 349). At hatching time, all the rudimentary sacral ribs are ossified in the chick, but only a few have a trace of ossification in the skua (*Maillard, 1948*); in the kiwi (*Apteryx oweni*) the ossification centers in the synsacral vertebral bodies and neural arches are still distinct (*Parker, 1892a*).

**The Caudal Vertebrae and the Pygostyle.** The development of the caudal vertebrae lags somewhat behind that of the more anterior vertebrae. In the 4-day gull (*Larus canus*) embryo (*Piiper, 1928*), for example, the perichordal tube and intercentra are found down to the base of the tail; but differentiation in the midportion of the tail has proceeded only to the stage of cranial and caudal sclerotomites and perichordal rings; at more caudal levels the sclerotomes are undivided, and the somites at the end of the tail are still saclike.

In the 5-day gull (*Larus canus*), the caudal vertebrae have reached the developmental stage seen in the cervical region of the 4-day embryo, that is, the inter- and intrasclerotomic fissures are obliterated and the perichordal rings have coalesced to form the perichordal tube. This condition exists to the end of the tail. The caudal intercentra are large and fill the entire space between the successive pairs of intersclerotomic arteries. After 6 days of incubation, the intercentra are even larger, especially in the midportion of the tail, and they are chondrified; in the ostrich (*Struthio camelus*), however, the intercentra atrophy in the caudal region as else-



where. The formation of secondary vertebral bodies has progressed to the posterior half of the 6-day gull embryo's tail, which, therefore, still lags 24 hours behind the cervical region. At the 8-day stage, it is possible to see that differentiation into vertebral and intervertebral bodies does not yet extend to the tip of the tail, although this region now consists of early cartilaginous tissue. The interdorsals and dorsal-interdorsals are considerably reduced in the caudal region. The dorsal portions of the basidorsals are directed craniad instead of caudad. The intervertebral bodies are larger than those at other levels. The neural arches are chondrified dorsally only in the anterior region of the tail (*Piiper, 1928*). In the kiwi (*Apteryx oweni*), all but the last two or three neural arches are completely chondrified at a stage that is equivalent to that of the chick's (*Gallus gallus*) eleventh or twelfth day (*Parker, 1892a*).

In the gull (*Larus canus*), the pygostyle embraces the last four or five caudal vertebrae. Their successive centra and neural arches have fused by the tenth day (*Piiper, 1928*). In the kiwis (*Apteryx australis*, *Apteryx oweni*) the pygostyle consists of only two or three fused vertebrae. In kiwi embryos corresponding to 8- or 9-day chicks, the body of the last caudal vertebra is a long cone of cartilage projecting far caudad of its neural arch; ventral to the notochord, it is fused with the body of the next more anterior vertebra. The neural arches in the region of the pygostyle are still open at the time of hatching, and their closure does not occur until the bird is almost full grown (*Parker, 1892a*).

At the end of incubation, ossification of the caudal vertebrae varies in degree in different birds (cf. Fig. 349); in some species, such as the pigeon (*Columba livia*), it has not even begun (*Schinz and Zangerl, 1937*). In the great skua (*Catharacta skua*), the process has not advanced beyond the stage of paired ventral ossification centers, which appear in succession in only the first five of the nine caudal vertebral bodies during the last 6 days of incubation (*Maillard, 1948*). In the chick, on the other hand, the last caudal vertebrae are already beginning to ossify on the nineteenth day, 2 days later than the most anterior caudal vertebrae (*Schinz and Zangerl, 1937*). In the kiwi (*Apteryx oweni*), also, ossification has extended to the end of the vertebral column by the hatching date (Fig. 350), although the intercentra, the diapophyses, the parapophyses, and the dorsal portions of the neural arches are still cartilaginous at that time (*Parker, 1892a*).

### *The True Ribs*

In birds, a variable number of thoracic vertebrae bear true ribs. These differ from the rudimentary ribs or costal processes of other vertebrae by their greater length and by their flexible articulation with the vertebrae. Also, the true ribs are each composed of a dorsal or vertebral portion,



which articulates with a vertebra, and a ventral or sternal portion, which articulates with the sternum. The dorsal portion of every true rib bears a short uncinatè process directed dorsocaudad. Anterior and posterior to the true ribs, there are a few so-called false ribs, which lack sternal segments.

Both vertebral and sternal portions of the true ribs have been generally assumed to originate from the sclerotomes of the somites. It is entirely possible, however, that only the vertebral portions are of this derivation, and that the sternal portions form from the mesoderm of the lateral plate (somatopleure). Straus and Rawles (1953) observed that powdered carbon placed on the trunk somites (the twenty-first to twenty-fifth) of 27- to 34-somite chick embryos always became localized in the vertebral segments of the ribs and in the musculature of the dorsal one third of the body, whereas carbon placed on the lateral plate appeared later in the sternal segments of the ribs and in other structures of the ventral one half to two thirds of the body wall.

The vertebral segments of the thoracic ribs form at approximately the same time and in the same manner as the costal processes of the cervical vertebrae. The true ribs begin their development as direct ventral processes of the vertebrae and are recognizable as such early in the seventh day of incubation, in the duck (*Anas platyrhynchos*) or the zebra parakeet (*Melopsittacus undulatus*) and at the middle or end of the sixth day, in the chick (Knopfli, 1919; Gladstone and Wakeley, 1932; Fell, 1939). At this time, both the vertebral and sternal portions consist of early pre-cartilage. The sternal segments are small, oval masses, each connected with a vertebral segment by a diffuse strand of mesenchyme. The ventral ends of the sternal segments are rounded and are in contact with, but sharply demarcated from, the diffuse bilateral mesodermal condensations that will give rise to the sternum. About 24 hours later, the proximal segments of the ribs are chondrified and the sternal segments are continuous with the sternal primordia (sternal plates). A similar stage of development is seen in the 21- to 23-mm. embryo of the European coot (*Fulica atra*) and in the 9-day herring gull (*Larus argentatus*) embryo (Knopfli, 1919). Fusion of the sternal rib segments with the sternum apparently begins with the more anterior ribs and progresses caudad (Fell, 1939).

In the 7- or 7.5-day chick, 8.5-day duck, 11-day herring gull, *Larus argentatus*, and 15-day emu, *Dromaeus novae-hollandiae* (Knopfli, 1919; Gladstone and Wakeley, 1932; Lutz, 1942), cartilage is replacing pre-cartilage in the sternal as well as in the vertebral portions of the ribs. The vertebral segments are directed caudad and the sternal segments craniad, so that an obtuse angle is enclosed between them. At this stage, syndesmotic joints exist between the two costal segments and between the



sternum and the ventral ends of the sternal segments; slightly later (that is, in the 8-day chick), a thick layer of perichondrium intervenes between the ribs and the sternum (*Gladstone and Wakeley, 1932*).

According to *Knopfli (1919)* the intercostal joints, which are clearly seen by the chick's eleventh day and the duck's (*Anas platyrhynchos*) twelfth, are formed through the atrophy of cartilage in the midst of the ribs, after the progressive chondrification of the latter from dorsal to ventral. This view has been questioned, however (*Lutz, 1942*), because of the observed independent chondrification centers in the sternal segments; it appears more likely that *Hoffmann (1879)* was correct in stating that the intercostal (and costosternal) joints result from the direct conversion of mesenchyme to connective tissue, without passing through a cartilaginous stage. The costosternal and costovertebral articulations form at approximately the same time as the intercostal joints (*Knopfli, 1919*).

The primordia of the uncinate processes are first seen about at the time when the sternal segments of the ribs start to become cartilaginous. Each process appears as a diffuse cell condensation behind the rib to which it will later be attached, at a relatively more dorsal level than it will occupy definitively (*Knopfli, 1919; Anasiewiczowna, 1928*). According to *Knopfli (1919)*, the uncinate processes become attached to the ribs on the chick's eighth day of incubation (or the duck's, *Anas platyrhynchos*, eleventh day); according to *Anasiewiczowna (1928)*, they begin to descend toward the ribs on the chick's ninth day and come into contact with them on the eleventh day. Chondrification of the uncinate processes begins on the chick's eighth or ninth day (*Knopfli, 1919; Anasiewiczowna, 1928*), and the duck's eleventh, and is extensive in both birds by the fifteenth day (*Knopfli, 1919*).

The vertebral and sternal divisions of the ribs ossify separately (*Schauinsland, 1906*). In the chick, ossification begins on the tenth (*Anasiewiczowna, 1928*) or twelfth day (*Knopfli, 1919*); in the duck (*Anas platyrhynchos*), its onset is relatively later, that is, not until the seventeenth day (*Knopfli, 1919*). The first part of the rib to ossify is the peripheral portion of the vertebral segment, above the attachment of the uncinate process. By the chick's fifteenth day, ossification has spread as far as the neck of the ribs, progressing inward also, and perichondral ossification is beginning ventral to the uncinate insertion. On the sixteenth day, the first indication of ossification is seen in the midpart of the sternal segments. By the day before hatching, the vertebral segments are almost entirely ossified, and peripheral ossification has spread throughout the sternal segments. In the duck, no ossification of the sternal segments is found as late as the twenty-sixth day of incubation.

The uncinate processes probably remain cartilaginous to the end of the incubation period (*Knopfli, 1919; Anasiewiczowna, 1928*), although some



indications of beginning ossification may be apparent on the chick's nineteenth or twentieth day, and the central portions of the processes are ossified in the newly hatched rhea, *Rhea americana* (Knopfli, 1919).

### The Skull

The skull consists of a large number of closely set and complexly inter-related bones. It is considered as being made up of two parts, an upper division, or neurocranium, which houses and protects the brain and the sense organs, and a separate lower portion, or viscerocranium, which is derived solely from the visceral arches and provides a framework for the lower jaw and the tongue.

The skull of birds resembles that of reptiles (especially crocodiles) in many particulars, among which may be mentioned the pneumatization of the bones and the presence of a single median occipital condyle articulating with the atlas. The plane of articulation, however, is not vertical but inclined backward, and in some species may be almost horizontal; also, the brain case is much larger than in the reptilian skull, in keeping with the increased size of the brain. Outstanding features of the avian cranium are the fusion of the bones, which occurs in most adult birds, and the magnitude of the orbits.

The neurocranium and the viscerocranium are each composed of both cartilage and membrane bones; the latter comprise the entire vault of the skull. The cartilage bones form in and replace the chondrocranium, or cartilaginous skull, which lies almost entirely beneath the brain and is a continuous structure produced during embryonic development by the fusion of separate cartilages. The ossification centers for the cartilage bones do not necessarily coincide with the chondrification centers. The membrane bones are exterior to the cartilage bones; some of them ossify more or less simultaneously with the latter, some earlier.

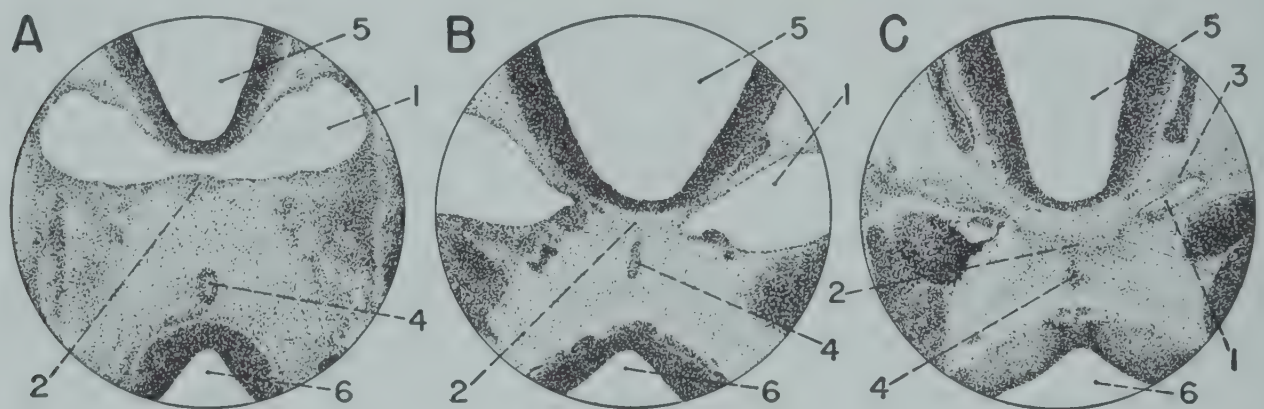
### The Chondrocranium

The cartilages that enter into the composition of the chondrocranium can be classified roughly into four groups. First, there are cartilages that form in close association with the notochord, including vestigial vertebrae that have been transferred from the vertebral column to the occipital region of the skull during the course of evolution; all these elements make up the basal plate, which comprises the base of the skull posterior to the hypophyseal region. The second group develops anterior to the rostral end of the notochord and on either side of the hypophysis and continues the base of the skull forward. The third group is composed of the cartilages that enclose or support the sense organs, and the fourth group of those that form in the visceral arches. The individual cartilages of these four groups enlarge, develop processes, and eventually come into contact and fuse



together, thus forming a structure of continuous cartilage, the chondrocranium.

**The Early Development of the Basis Cranii.** The first cartilages of the chondrocranium to appear are those of the basal plate, which develop in proximity to the chorda. The earliest to form is the acrochordal cartilage or plate; this is also the first cartilaginous element of the whole skeleton (*Parker, 1892a; Sonies, 1907; Beer and Barrington, 1934*). The acrochordal cartilage received its name because of its position at or near the anterior end of the chorda. Its median portion will form the dorsum sellae, the posterior boundary of the hypophyseal fossa.



**Fig. 351.** Early formation of cartilage in the occipital region of the skull, depicting the filling of the head cavities with the mesenchyme of the acrochordal cartilage, in the pro-otic region of the duck embryo (*Anas platyrhynchos*) between 3.5 and 4.5 days. (Redrawn with modifications after Jager, 1926.)

A, head cavities are joined by hollow transverse commissure, with the acrochordal cartilage in a prochondral stage; B, commissure has broken down, and the acrochordal cartilage appears as a condensation localized between the two cavities; C, the head cavities have almost disappeared, and the anlage of the acrochordal cartilage is larger.

1, head cavity; 2, acrochordal cartilage; 3, lamina antotica; 4, notochord; 5, dien-cephalon; 6, mesencephalon.

As Jager (1926) showed, the acrochordal cartilage arises in connection with the transverse mesodermal commissure (hollow in the duck, *Anas platyrhynchos*) that joins the premandibular head cavities or first pro-otic somites. In the duck embryo incubated about 4 days or slightly longer, a mesodermal condensation ventral to the transverse commissure represents the earliest anlage of the acrochordal cartilage (Fig. 351-A). The chorda, which bends ventrad in passing from the cervical to the cranial region, extends to the vicinity of the mesodermal condensation. During the fifth day (Jager, 1926) or early in the sixth (Sonies, 1907), the transverse commissure disappears, and the head cavities are now connected only by the acrochordal anlage which surrounds the end of the notochord (Fig. 351-B and C). The head cavities disappear during the course of the duck's sixth day (Jager, 1926).

During the duck's (*Anas platyrhynchos*) sixth day and the chick's fifth



(Sonies, 1907; Beer and Barrington, 1934), the acrochordal cartilage is seen as a narrow, flat, transversely elongated bar lying in the frontal plane near the rostral end of the chorda (Fig. 352-A<sub>2</sub>). It is also present in the 43-mm. embryo of the jackass penguin, *Spheniscus demersus* (Crompton, 1953). The tip of the chorda emerges from the ventral side of the cartilage and bends ventrad, to end behind the hypophysis. The acrochordal

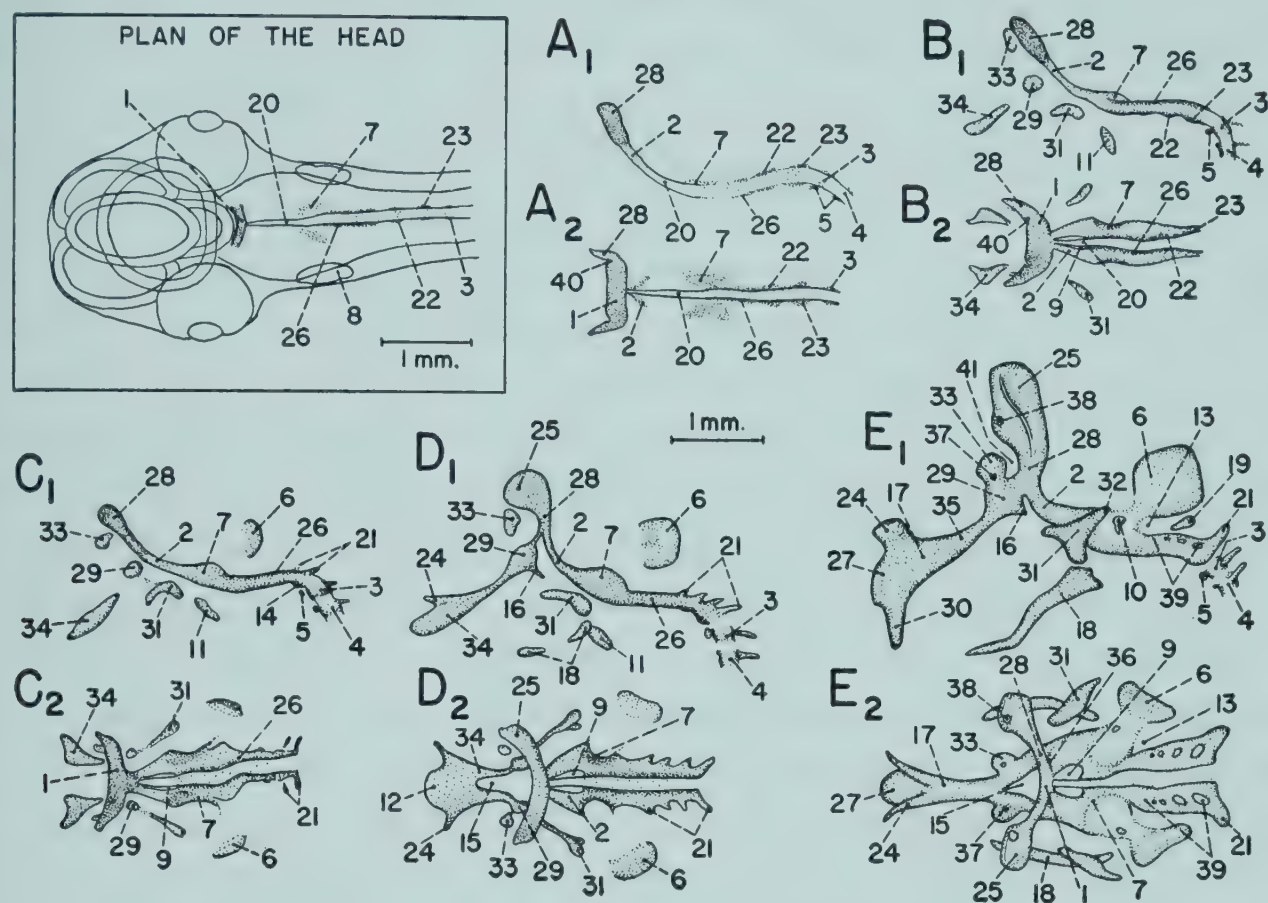


Fig. 352. Successive stages in the development of the chondrocranium of the duck embryo (*Anas platyrhynchos*) from the first appearance of cartilages in the occipital region at 5.5 days to the end of the eighth day. (Redrawn with modifications after Beer and Barrington, 1934.)

Insert: dorsal view of head at 5.5 days, showing cartilages and vertebrae in relation to the notochord.

A<sub>1</sub>, A<sub>2</sub>, lateral and dorsal views of chondrocranium at 5.75 days; B<sub>1</sub>, B<sub>2</sub>, lateral and dorsal views of same at 6 days; C<sub>1</sub>, C<sub>2</sub>, lateral and dorsal views of same at 6.5 days, when cartilages have appeared in the ethmoid region; D<sub>1</sub>, D<sub>2</sub>, lateral and dorsal views of same at 7 days; E<sub>1</sub>, E<sub>2</sub>, lateral and dorsal views of same at 8 days. All in scale.

1, acrochordal cartilage; 2, anterior parachordal cartilage; 3, atlas; 4, axis; 5, hypocentra of atlas and axis; 6, pars canaliculi of auditory capsule; 7, pars cochlearis of auditory capsule; 8, auditory sac; 9, basicranial fenestra; 10, columella auris; 11, copula; 12, ethmoid plate; 13, fissura metotica; 14, hypocentra of occipital vertebrae; 15, hypophyseal fenestra; 16, infrapolar process; 17, interorbital septum; 18, Meckel's cartilage; 19, metotic cartilage; 20, notochord; 21, occipital arch of segments 6 to 9; 22, first occipital vertebra; 23, second occipital vertebra (proatlas); 24, orbital cartilage, anterior portion; 25, orbital cartilage, posterior portion; 26, parachordal cartilage; 27, parietotectal cartilage of the nasal capsule; 28, pila antotica; 29, polar cartilage; 30, prenasal process; 31, quadrate; 32, otic process of quadrate; 33, suprapolar cartilage; 34, trabecula cranii; 35, trabecula communis; 36, foramen abducens; 37, foramen for the ophthalmic artery; 38, foramen trochlearis; 39, foramina for hypoglossal nerve roots; 40, notch for abducens nerve; 41, notch for oculomotor nerve.



cartilage of the chick is somewhat larger anteroposteriorly than that of the duck (Sonies, 1907).

The second element to appear, the parachordal, is identifiable only slightly later than the acrochordal, that is, about midway through the chick's fourth incubation day (Jager, 1926) and the duck's (*Anas platyrhynchos*) sixth (Beer and Barrington, 1934). In the beginning, the parachordal is entirely separate from the acrochordal (Sonies, 1907) and lies some distance behind it, surrounding the notochord from a level opposite the middle of the otic vesicle halfway to the atlas sclerotome (cf. Fig. 352-A<sub>1</sub> and A<sub>2</sub>). In birds, with the observed exception of the kiwi, *Apteryx oweni* (Parker, 1892a, 1892b), the parachordal apparently arises as an unpaired structure; it has the form of a cylinder, which, however, is thicker laterally than elsewhere (Froriep, 1883; Sonies, 1907).

Between the parachordal and the atlas (cf. Fig. 352-A<sub>1</sub> and A<sub>2</sub>) the notochord is enveloped by two sclerotomic rings (Sonies, 1907; Jager, 1926; Beer and Barrington, 1934). The cranial and caudal sclerotomites, the perichordal ring, and the intercentrum of the more posterior sclerotome are clearly visible; those of the more anterior are not so easily identifiable (Beer and Barrington, 1934). The differentiation of these structures is a clear indication that the posterior end of the base of the skull will be formed of two included vertebrae and that the occipitoatlantal joint in birds is intervertebral, that is, intrasclerotomic. Additional evidence of segmentation in the basal plate as seen in the kestrel, *Falco tinnunculus* (Suschkin, 1898), and the 4- or 5-day chick embryo (Froriep, 1883; Jager, 1926), consists of the presence of four pairs of myotomes between the vagus nerves and the first pair of spinal ganglia. Since each vagus nerve appears at the level of the erstwhile second metotic somite, it is apparent that the four occipital segments correspond to the third to sixth pairs of metotic somites, or the sixth to ninth somites of the entire series. From the circumstance that the occipitoatlantal joint is intrasclerotomic, it follows that there are nine and one-half segments in the bird's skull (Beer and Barrington, 1934).

Before the end of the day on which they appear, the two occipital vertebrae become synchondrosial with each other and with the parachordal (Sonies, 1907; Jager, 1926; Beer and Barrington, 1934), which is thus lengthened caudad (Fig. 352-B<sub>2</sub>). The vertebral portion of the parachordal is still distinguishable, however, for the two intercentra project ventrally (Fig. 352-B<sub>1</sub> and C<sub>1</sub>) as late as the chick's sixth day of incubation (Froriep, 1883) and the duck's (*Anas platyrhynchos*) eighth (Beer and Barrington, 1934).

Almost from the moment of its appearance, the parachordal is flanked at its anterior end by a pair of elements that are the anlagen of the cochlear portions of the auditory capsule (Fig. 352-A<sub>1</sub> and A<sub>2</sub>). Each pars cochlearis, also called the basiotic or mesotic cartilage (Sonies, 1907), is an elongated

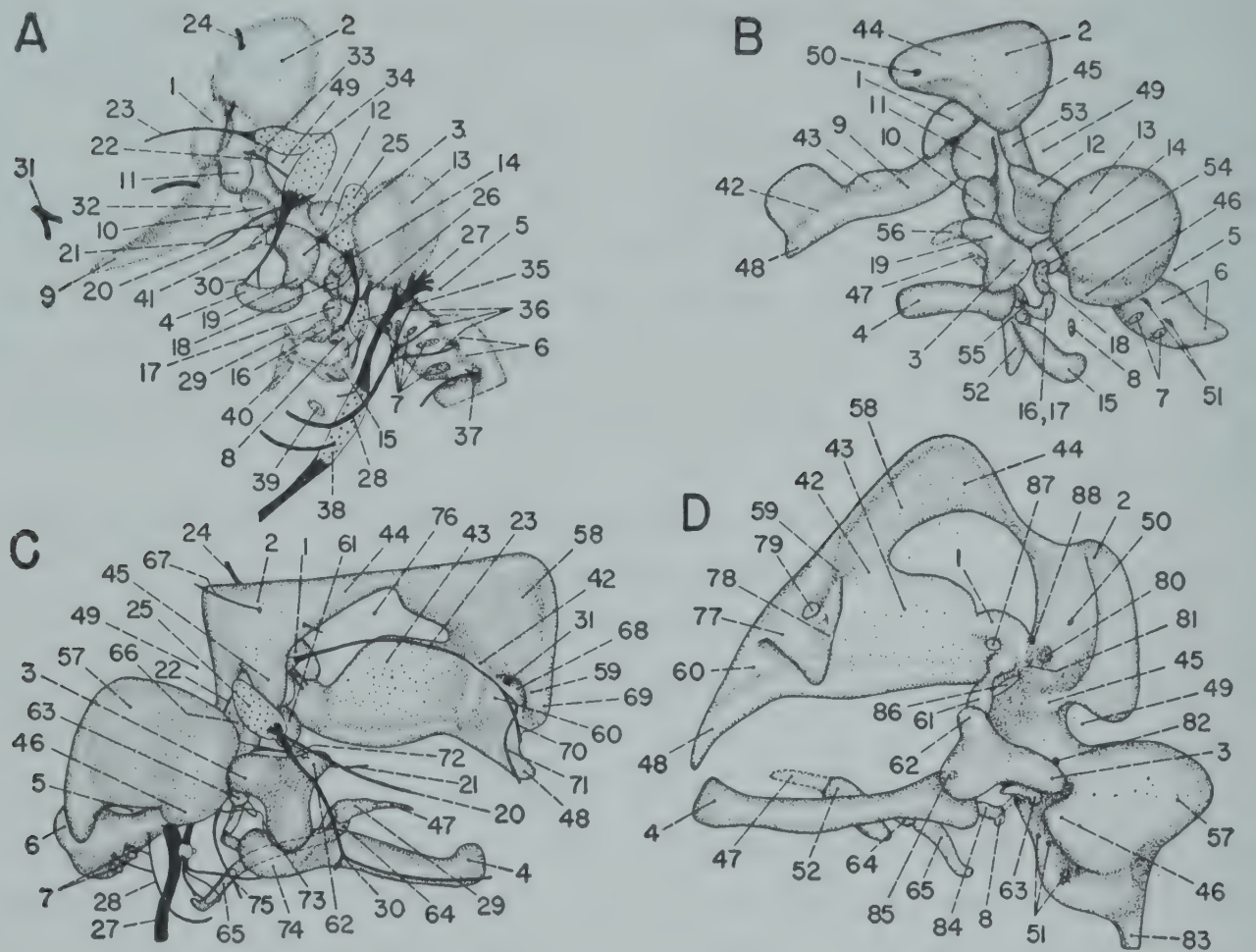


curved structure applied against the convex medial surface of the cochlear portion of the auditory sac. In the duck, *Anas platyrhynchos* (Beer and Barrington, 1934), the pars cochlearis arises close to but quite separate from the parachordal and first contacts the latter about at the time of its fusion with the occipital vertebrae (that is, before the end of the sixth day); cf. Fig. 352-B<sub>2</sub>. Observers of the chick (Sonies, 1907) and the kiwi, *Apteryx oweni* (Parker, 1892b), have stated that the pars cochlearis is an integral part of the parachordal from the beginning or at least chondrifies in continuity with it.

About at the time of fusion of the parachordal with the occipital vertebrae and the pars cochlearis, the parachordal, together with the notochord begins to curve downward beneath the rhombencephalon, and the anterior end of the notochord starts to curve upward (Sonies, 1907; Lang, 1952). As a result, the formerly dorsal surface of the acrochordal is turned somewhat toward the rear (cf. Fig. 342-B<sub>1</sub>; Fig. 354-A<sub>1</sub>, B<sub>1</sub>, and C<sub>1</sub>). The acrochordal cartilage now enlarges slightly and at each lateral end has developed an upward and anteriorly directed process (cf. Fig. 352-B<sub>2</sub>), the anlage of the pila or lamina antotica (Beer and Barrington, 1934). Sonies (1907) believed that the pila antotica forms independently of the acrochordal in the chick. The abducens nerve passes out through a notch (later a foramen) located at the junction of the medial edge of the pila antotica with the main part of the acrochordal (Beer and Barrington, 1934); in the penguin (*Spheniscus demersus*), however, this nerve passes out behind the pila antotica, between the latter and the pars cochlearis (Crompton, 1953).

In the duck (*Anas platyrhynchos*) an additional pair of elements has been seen at this time proceeding from the posterior edge of the acrochordal on either side of the notochord and diverging from it posteriorly as they approach the cochlear capsules (cf. Fig. 352-B<sub>2</sub>). These new elements were named the anterior parachordal cartilages by Beer and Barrington (1934), who first described them. By the end of the duck's sixth day of incubation, the anterior parachordals have fused with the widened parachordal cylinder and with the cochlear capsules, so that all the elements of the basal plate are united (Fig. 352-C<sub>1</sub>). A somewhat different account of the unification of the basal plate has been given by other writers; Sonies (1907), for example, stated that, in the chick, the pars cochlearis grows forward in continuity with the parachordals and fuses with the acrochordal. Anteriorly, the basal plate is pierced by the posterior basicranial fenestra (cf. Fig. 352-C<sub>2</sub>), which, in the duck, lies at the level of the anterior parachordals (Beer and Barrington, 1934). The notochord traverses this space. No posterior basicranial fenestra forms in the penguin, *Spheniscus demersus*, or the emu, *Dromaeus novae-hollandiae* (Crompton, 1953). In the chick (*Gallus gallus*), the basicranial fenestra has been said to result from the resorption of cartilage (Gaupp, 1905) and from the secondary separation of





**Fig. 353.** The chondrocranium of the penguin (*Spheniscus demersus*) at various stages of development, shown in lateral view. (Redrawn with modifications after Crompton, 1953.)

A, left side, at 43-mm. stage ( $\times 10$ ); B, the same, at 47-mm. stage ( $\times 10$ ); C, right side, at 56-mm. stage ( $\times 8$ ); D, left side, at 58-mm. stage ( $\times 6$ ).

1, suprapolar cartilage; 2, postorbital cartilage; 3, processus oticus; 4, Meckel's cartilage; 5, incisura metotica; 6, occipital arch; 7, cranial ribs; 8, pharyngobranchial; 9, trabeculae; 10, anlage of the basitrabecular process; 11, polar cartilage; 12, pars cochlearis; 13, pars canicularis; 14, pharyngohyal; 15, first branchial arch; 16, hypohyal; 17, ceratohyal; 18, epihyal; 19, pars quadrata; 20, ramus maxillaris; 21, ramus palatinus; 22, abducent nerve; 23, ramus profundus; 24, oculomotor nerve; 25, ganglion geniculatum; 26, glossopharyngeal nerve; 27, vagus plus accessory nerve; 28, hypoglossal nerve; 29, first copula; 30, ramus mandibularis; 31, olfactory nerve; 32, lateral carotid incisure; 33, acrochordal cartilage; 34, ganglion Gasseri; 35, ganglion petrosum; 36, hypoglossal roots; 37, first spinal nerve; 38, ganglion jugulare; 39, second branchial arch; 40, second copula; 41, blastematous pars pterygoidea; 42, preoptic root of the orbital cartilage; 43, interorbital septum; 44, supraorbital cartilage; 45, pila antotica spuria; 46, metotic cartilage; 47, processus lingualis; 48, prenasal process; 49, incisura pro-otica; 50, foramen for the trochlear nerve; 51, hypoglossal foramen; 52, copula; 53, lateral abducent commissure; 54, processus supracolumellaris lateralis; 55, blastematous connection between the hyoid arch and the first copula; 56, blastematous pars pterygoidea; 57, auditory capsule; 58, anterior orbital cartilage; 59, sphenethmoid commissure; 60, nasal septum; 61, basitrabecular process; 62, processus orbitoquadratus; 63, columella auris; 64, ceratobranchial; 65, epibranchial; 66, processus lateralis partis cochlearis; 67, trochlearis nerve; 68, foramen olfactorium evehens; 69, parietotectal cartilage; 70, ramus lateralis nasi; 71, ramus medialis nasi; 72, blastematous tract connecting the processus basitrabecularis with the pars quadrata; 73, chorda tympani; 74, processus retroarticularis; 75, hyomandibular nerve; 76, sphenoid fontanelle; 77, nasal capsule side wall; 78, planum antorbitale; 79, foramen for the ramus medialis nasi; 80, foramen for the profundus nerve; 81, foramen for the abducent nerve; 82, foramen for the facial nerve; 83, anlage of the tectum



the originally paired parachordals in the middle of the second week of incubation (*Parker, 1869*).

In the 6-day chick or 6.5-day duck (*Anas platyrhynchos*) embryo (*Froriep, 1883; Sonies, 1907; Beer and Barrington, 1934*), two pairs of occipital arches can be seen extending dorsad from the posterior end of the basal plate (Fig. 352-C<sub>1</sub> and C<sub>2</sub>). These are the neural arches of the two included occipital vertebrae. In the 7-day duck, two pairs of smaller occipital arches have appeared anterior to the first two, at the level of the posterior part of the originally continuous parachordal cylinder (Fig. 352-D<sub>1</sub> and D<sub>2</sub>). A pair of hypoglossal nerve roots emerges anterior to each of the last three pairs of arches (the most cranial of the four pairs of ventral nerve roots has disappeared during the duck's fifth day). By the middle of the eighth day (Fig. 352-E<sub>1</sub> and E<sub>2</sub>), the successive occipital arches on each side have fused with one another dorsal and lateral to the nerve roots, which are thus enclosed in foramina (*Beer and Barrington, 1934*). The fourth arch and the third foramen, although not present in the 43-mm. penguin (*Spheniscus demersus*), have appeared in the 47-mm. embryo (*Crompton, 1953*), and they are present in the 15-day emu (*Dromaeus novae-hollandiae*) embryo (*Lutz, 1942*). Three hypoglossal foramina, implying the presence of four arches, have also been seen in embryos of the sparrow, *Passer domesticus*, and the starling, *Sturnus vulgaris* (*Sonies, 1907*). The lateral halves of the arches never fuse dorsally with their counterparts.

Vestigial ribs now appear lateral and ventral to the basal plate. The cranial ribs are represented by a series of paired mesenchymatous condensations alternating with the roots of the hypoglossal nerve; the last pair of condensations is associated with the intercentrum of the second (posterior) occipital vertebrae. Three pairs of occipital ribs have been seen in the 6- to 8-day chick embryo (*Froriep, 1883*) and in the embryo of the kestrel, *Falco tinnunculus* (*Suschkin, 1896*); four pairs have been counted in the 7.5-day duck (*Beer and Barrington, 1934*); and five pairs have been found in the 43-mm. penguin, *Spheniscus demersus* (*Crompton, 1953*), as Fig. 353 shows. These ribs disappear without chondrifying (*Sonies, 1907*); only the two posterior pairs remain in the 56-mm. penguin, and none are identifiable in the 58-mm. embryo (*Crompton, 1953*).

The posterior edge of the basal plate forms the ventral border of the foramen magnum, and the centrum of the second occipital vertebra serves as the occipital condyle. The paired elements of this centrum are seen in the 9-day duck (*Anas platyrhynchos*) as prominences flanking the notochord (*Beer and Barrington, 1934*).

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synoticum; 84, stylohyal cartilage; 85, groove in the pars quadrata for the quadratojugal; 86, lateral carotid foramen; 87, foramen for the ophthalmic artery; 88, foramen for the oculomotor nerve.



**The Early Development of the Prechordal Region.** The first cartilages to appear in front of the rostral end of the notochord are the trabeculae cranii, which are seen in the chick or duck (*Anas platyrhynchos*) embryo incubated 6 days (Sonies, 1907; Beer and Barrington, 1934). These elements are elongated cartilaginous bars, wider anteriorly than posteriorly, lying approximately parallel to each other beneath the forebrain (cf. Fig. 352-B<sub>1</sub>



Fig. 354. Developmental changes in the relationship of the median part of the chondrocranium to the brain, and of the prehypophyseal and posthypophyseal portions of the chondrocranium to each other, shown by median sagittal sections of embryos of three avian species. (Redrawn with modifications after Lang, 1952.)

A<sub>1</sub> to A<sub>5</sub>, zebra parakeet (*Melopsittacus undulatus*) of 2.5-mm., 5-mm., 7-mm., 8.6-mm., and 11-mm. head length; B<sub>1</sub> to B<sub>5</sub>, chicken (*Gallus gallus*) of 2.5-mm., 7-mm., 13-mm., 13.5-mm., and 17-mm. head length; C<sub>1</sub> to C<sub>5</sub>, pigeon (*Columba livia*) of 2.7-mm., 7-mm., 8-mm., 8.5-mm., and 15-mm. head length.

Cartilage shown in stippling, brain and ossified regions in black.

1, notochord; 2, acrochordal cartilage; 3, trabeculae; 4, polar cartilage; 5, infrapolar process; 6, parachordal cartilage; 7, ethmoidal region; 8, interorbital septum; 9, basisphenoid; 10, basitemporal; 11, rostrum parasphenoidei; 12, basioccipital; 13, cerebral hemispheres; 14, diencephalon; 15, hypophysis; 16, optic lobes; 17, rhombencephalon; 18, cerebellum; 19, premaxillary; 20, nasal; 21, frontal.

and B<sub>2</sub>). In the beginning, they are entirely independent of the acrochordal, and their long axes make an angle of about 90° with the anterior part of the basal plate (cf. Fig. 352-C<sub>2</sub>); this angle gradually increases to 160° or more during the course of development, depending upon the species (Fig. 354).

Behind the posterior ends of the trabeculae, between them and the



acrochordal cartilage, are the paired polar cartilages (Sonies, 1907; Beer and Barrington, 1934). These arise as small spheroidal nodules (cf. Fig. 352-B<sub>1</sub>, C<sub>1</sub>, and C<sub>2</sub>). They form independently in the duck, *Anas platyrhynchos*, the starling, *Sturnus vulgaris* (Sonies, 1907), and the penguin, *Spheniscus demersus* (Crompton, 1953), but they presumably chondrify in continuity with the trabeculae in the chick (Sonies, 1907).

Dorsal to the polar cartilages and anteroventral to the pila antotica is another pair of similar elements (cf. Fig. 352-B<sub>1</sub> and C<sub>1</sub>), the supratrabecular (Suschkin, 1896, 1898) or suprapolar cartilages (Sonies, 1907; Beer and Barrington, 1934). Sonies (1907) could not identify these as independent elements in the chick.

A third pair of nodules, the blastematous anlagen of the basitrabecular processes, are present in the 43-mm. penguin (*Spheniscus demersus*) embryo ventrolateral to the polar cartilages (cf. Fig. 353-A); each of them is syndesmotically connected with the medial anterodorsal surface of the quadrate cartilage until the 61-mm. stage (Crompton, 1953). The basitrabecular process is also present in the kiwi, *Apteryx oweni* (Parker, 1892a), the swallow, *Hirundo rustica* (Beer and Barrington, 1934), the 13.5-mm. ostrich, *Struthio camelus* (Brock, 1937), and the emu, *Dromaeus novae-hollandiae* (Lutz, 1942). It does not form in the duck (*Anas platyrhynchos*) but, according to Beer and Barrington (1934), is represented in the 6.5-day embryo by a very transitory membranous connection between the polar and quadrate cartilages (cf. Fig. 352-C<sub>1</sub> and C<sub>2</sub>). In the ostrich, *Struthio camelus* (Brock, 1937), and the emu, *Dromaeus novae-hollandiae* (Lutz, 1942), the entire basitrabecular process chondrifies. In the penguin (*Spheniscus demersus*), all but its distal part, which never chondrifies, has become cartilaginous by the 47-mm. stage (Crompton, 1953). The process here termed the basitrabecular has also been called the basipterygoid and the basipolar.

Late in the duck's (*Anas platyrhynchos*) seventh incubation day (cf. Fig. 352-D<sub>1</sub> and D<sub>2</sub>), each polar cartilage fuses with the posterior end of the trabecula of the same side (Sonies, 1907; Beer and Barrington, 1934). This fusion has occurred in the 13.5-mm. ostrich, *Struthio camelus* (Brock, 1937). In the penguin (*Spheniscus demersus*), the polar cartilages are continuous with but demarcated from the trabeculae at the 43-mm. stage (cf. Fig. 353-A) and have fused indistinguishably with them and with the basitrabecular process by the 56-mm. stage (Crompton, 1953).

The polar cartilages fuse with the acrochordal at the beginning of the seventh day in the chick (Sonies, 1907) and at its end in the duck, *Anas platyrhynchos* (Fig. 352-D<sub>1</sub>, D<sub>2</sub>, and E<sub>1</sub>); this fusion is incipient in the 13.5-mm. ostrich, *Struthio camelus* (Brock, 1937), and has already taken place in the 43-mm. penguin, *Spheniscus demersus* (Crompton, 1953), and the 11-mm. starling, *Sturnus vulgaris* (Sonies, 1907). The hypophyseal



foramen is thus delimited laterally. The internal carotid artery passes medialward through the space—the lateral carotid incisure—bounded by the acrochordal, the trabecula, and the polar cartilage. From the ventroposterior surface of each polar cartilage, the infrapolar process extends toward the basal plate (cf. Fig. 352- $D_1$  and  $E_1$ ); however, in the ostrich, *Struthio camelus* (Brock, 1937), this process is of negligible size, and it does not form in the penguin (Crompton, 1953). It appears earlier and is more pronounced in the starling and the sparrow (*Passer domesticus*) than in the duck and chick (Sonies, 1907).

During the latter half of the duck's (*Anas platyrhynchos*) seventh day of incubation, the anterior ends of the trabeculae become connected across the midline by the ethmoid plate (cf. Fig. 352- $D_1$  and  $D_2$ ), which chondrifies in continuity with the trabeculae (Sonies, 1907). The formation of the ethmoid plate creates a rostral boundary for the hypophyseal foramen, which is separated from the posterior basicranial foramen by the acrochordal cartilage. In the penguin (*Spheniscus demersus*), however, the hypophyseal foramen is bounded posteriorly by the precarotid commissure (Fig. 355-A), which connects the posterior ends of the trabeculae in the 43-mm. embryo and forms the anterior boundary of the median foramen for the internal carotid artery (Crompton, 1953). It is possible that a similar precarotid commissure forms in the emu, *Dromaeus novae-hollandiae* (Crompton, 1953), and the kiwi, *Apteryx oweni* (Parker, 1892a). In most birds, the internal carotid artery passes through the hypophyseal foramen.

In the chick, the anterior ends of the trabeculae themselves fuse together by the beginning of the seventh day of development, forming the trabecula communis (Sonies, 1907). In the 7.5-day duck (*Anas platyrhynchos*), the formation of the trabecula communis in the midline behind the ethmoid plate reduces the anterior extent of the hypophyseal foramen (cf. Fig. 352- $E_1$ ). The trabecula communis is present in the 47-mm. penguin, *Spheniscus demersus* (Fig. 355-B), and the 15-day emu (*Dromaeus novae-hollandiae*) embryo (Lutz, 1942). In the kestrel, *Falco tinnunculus* (Suschkin, 1898), and the pigeon, *Columba livia* (Filatoff, 1906), the fusion of the trabeculae is said to be brought about through the mediation of an independent intertrabecula formed between the trabeculae. From either the trabecula communis or the intertrabecula arise the interorbital septum, the nasal septum, and the prenasal process.

In the 7.5- or 8-day duck (*Anas platyrhynchos*), and the ostrich (*Struthio camelus*) embryo of 10.5-mm. head length (Brock, 1937), the suprapolar cartilage has fused with the polar cartilage anterior and posterior to the ophthalmic artery (Sonies, 1907; Beer and Barrington, 1934), which is thereby enclosed in a foramen (cf. Fig. 352- $E_1$ ). This foramen is present in the 56-mm. penguin, *Spheniscus demersus* (Fig. 355-C), but its anterior wall is formed by the fusion of the suprapolar cartilage with a posteriorly



directed process of the trabecula rather than with the polar cartilage (Crompton, 1953). In the chick, the ophthalmic foramen is delimited dorsally by a semicircular strip of cartilage, which appears to be the homologue of the suprapolar cartilage in this species (Sonies, 1907).

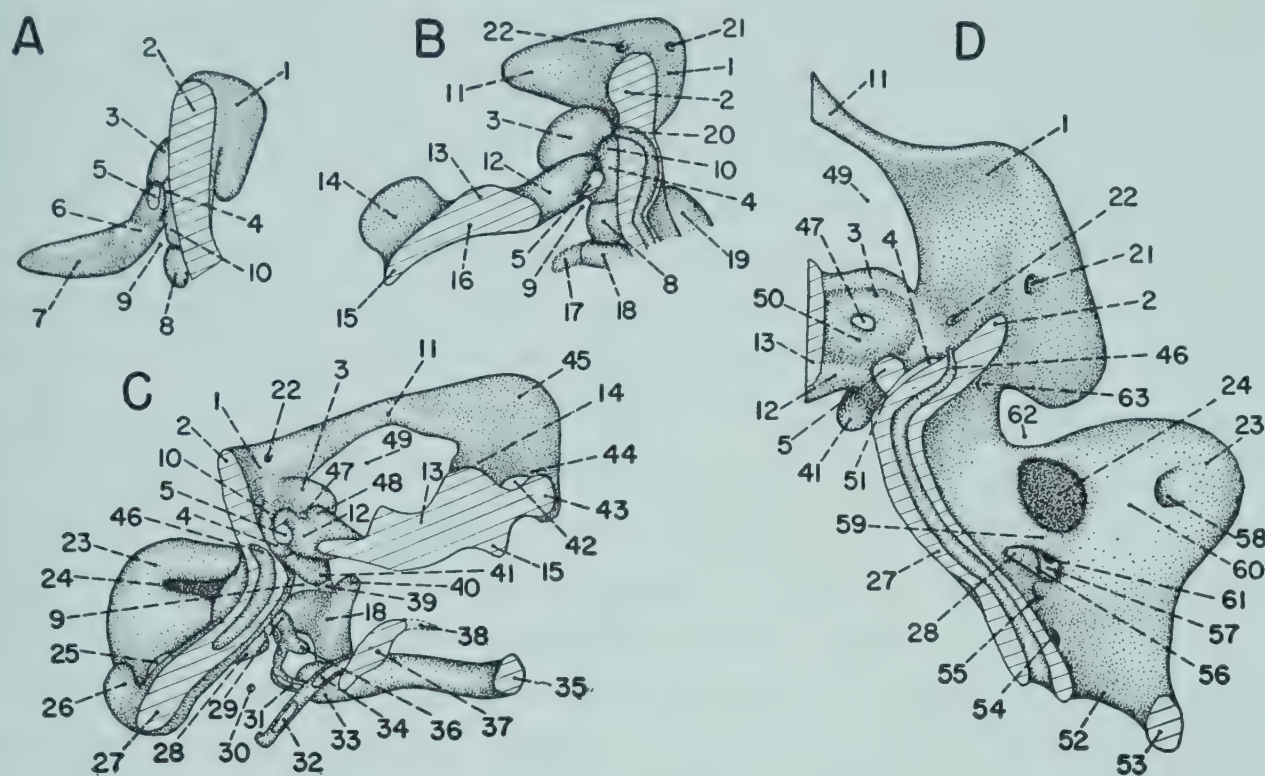


Fig. 355. Medial views of the chondrocranium of the penguin embryo (*Spheniscus demersus*) at various stages, shown by sagittal sections. (Redrawn with modifications after Crompton, 1953.)

A, the right half of the prechordal region and the anterior part of the basal plate at the 43-mm. stage; B, similar view at the 47-mm. stage; C, the left half of the entire chondrocranium at the 56-mm. stage; D, the right half of the chondrocranium, anterior portion removed, at the 58-mm. stage. All  $\times 10$ .

1, postorbital cartilage; 2, acrochordal cartilage; 3, suprapolar cartilage; 4, foramen caroticum; 5, precarotid commissure; 6, notch for Rathke's pouch; 7, trabeculae; 8, anlage of the basitrabecular process; 9, lateral carotid incisure; 10, polar cartilage; 11, supraorbital cartilage; 12, foramen hypophyseos; 13, interorbital septum; 14, preoptic root of the orbital cartilage; 15, prenasal process; 16, trabecula communis; 17, blastematous pars pterygoidea; 18, pars quadrata; 19, pars cochlearis; 20, notochord; 21, foramen for the trochlear nerve; 22, foramen for the oculomotor nerve; 23, auditory capsule; 24, foramen acusticum; 25, incisura metotica; 26, occipital arch; 27, basal plate; 28, metotic cartilage; 29, columella auris; 30, pharyngohyal; 31, stylohyal cartilage; 32, epibranchial; 33, processus retroarticularis; 34, ceratobranchial; 35, Meckel's cartilage; 36, processus extracolumellaris; 37, copula; 38, processus lingualis; 39, blastematous tract connecting the processus basitrabecularis with the pars quadrata; 40, processus orbitoquadratus; 41, basitrabecular process; 42, foramen olfactorium evehens; 43, nasal septum; 44, sphenethmoid commissure; 45, anterior orbital cartilage; 46, groove lodging the notochord; 47, foramen for the ophthalmic artery; 48, posterodorsally directed process of the trabeculae; 49, sphenoid fontanelle; 50, hypophyseal pit; 51, lateral carotid foramen; 52, foramen magnum; 53, anlage of the tectum synoticum; 54, hypoglossal foramen; 55, foramen for the vagus nerve; 56, recessus scalae tympani; 57, ventral cochleo-canalicular commissure; 58, foramen for the ductus endolymphaticus; 59, dorsal cochleo-canalicular commissure; 60, pre-endolymphatic commissure; 61, foramen perilymphaticum; 62, incisura pro-otica; 63, foramen for the abducent nerve.



**The Auditory Capsule.** The auditory capsule is formed by the fusion of two originally separate chondrifications, the pars cochlearis and the pars canalicularis; as their names imply, the former arises in proximity to the cochlea, or lagena, and the latter develops close to the semicircular canals. They are separated from the more anterior parts of the chondrocranium by the wide incisure pro-otica, which lodges the Gasserian ganglion and is traversed by all branches of the trigeminal nerve (cf. Fig. 353-A). The pars cochlearis appears very early and is an integral part of the basal plate almost from its beginning. The pars canalicularis is first seen during the chick's sixth day of development and the duck's (*Anas platyrhynchos*) seventh (cf. Fig. 352-D<sub>1</sub>), or less than 24 hours later than the pars cochlearis (Sonies, 1907; Beer and Barrington, 1934). Sonies (1907) observed that it chondrifies in the duck from a center located between the superior and lateral semicircular canals. It first covers the lateral surfaces of these canals and the lateral and ventral surfaces of the posterior semicircular canal, then spreads over the dorsal surface of the superior canal, the ventral surface of the lateral canal, and the caudal surface of the posterior canal. Soon it acquires the shape of a cup, with a dorsomedial opening for the passage of the acoustic nerve; this is the form of the pars canalicularis in the 43-mm. penguin (*Spheniscus demersus*) embryo (Crompton, 1953). Meantime, as the basal plate widens, the pars cochlearis grows laterally and somewhat upward toward the pars canalicularis. An anterior and a posterior cartilaginous connection then form between the two parts; the posterior connection is present in the 7.5-day duck (cf. Fig. 352-D<sub>1</sub> and D<sub>2</sub>) and both have formed in the 8-day duck (Fig. 352-E<sub>2</sub>), the 6.5-day chick (Sonies, 1907), and the 56-mm. penguin, *Spheniscus demersus*, as shown in Fig. 356-A<sub>1</sub> (Crompton, 1953).

Dorsomedially, the cartilaginous connections between partes cochlearis and canalicularis remain separated by a large space, the foramen for the acoustico-facial nerve (cf. Fig. 356-A<sub>1</sub>). This opening is soon subdivided into the large posterior acoustic foramen and the smaller anterior facial foramen; somewhat later, in the 58-mm. penguin (*Spheniscus demersus*), the elongated acoustic foramen is reduced to a round aperture as a cartilaginous commissure grows across it to transform its posterior end into a foramen for the endolymphatic duct (cf. Fig. 355-D). In the 74-mm. penguin (Crompton, 1953), as in the 10-day chick (Tonkoff, 1900b), the acoustic foramen has been further subdivided into four foramina for the various rami of the nerve, but the extent of the former aperture is indicated by a depression lodging the geniculate ganglion (Fig. 357-A<sub>2</sub>).

In the chick and the duck (*Anas platyrhynchos*), there is also a ventrolateral space between the commissures connecting the two parts of the otic capsule. This space apparently represents the primitive fenestra ovalis (Sonies, 1907; Beer and Barrington, 1934). In the 8.5-day duck, an addi-



tional ventral commissure between the partes cochlearis and canalicularis has delimited the perilymphatic foramen, which is bordered dorsally by the dorsal commissure, ventrally by the ventral commissure, medially by the pars cochlearis, and laterally by the pars canalicularis (Fig. 356-A<sub>2</sub>). In the penguin, *Spheniscus demersus* (Crompton, 1953), and the ostrich, *Struthio camelus* (Brock, 1937), no defect remains in the lateral wall of the otic capsule, and the fenestra ovalis is apparently a secondary formation resulting from atrophy of cartilage around the footplate of the columella auris. In the penguin, the ventral commissure and the perilymphatic foramen appear earlier than the fenestra ovalis (Crompton, 1953).

Behind and below the otic capsule is a space, the incisura or fissura metotica, which is bounded ventrally by the basal plate and posteriorly by the prominent occipital arch (cf. Fig. 352-E<sub>1</sub> and E<sub>2</sub>; Fig. 353-A and B). The glossopharyngeal and vagus nerves pass out through this space. Except for foramina for these nerves, most of the incisura metotica is soon obliterated by the fusion of the otic capsule with the occipital arch, the basal plate, and a third element, the metotic cartilage.

The development and role of the metotic cartilage seems to vary somewhat in different species of birds. In the duck (*Anas platyrhynchos*) and the chick, this cartilage appears as an independent chondrification (cf. Fig. 352-E<sub>1</sub>) lateral to the incisura metotica (Sonies, 1907; Beer and Barrington, 1934) and grows forward, fusing first with the basal plate and then with the otic capsule, although, in the chick, it sometimes fuses with the otic capsule first (Sonies, 1907); in the kiwi, *Apteryx oweni* (Parker, 1892a), the ostrich, *Struthio camelus* (Brock, 1937), and the penguin, *Spheniscus demersus* (Crompton, 1953), it arises in continuity with the pars canalicularis and grows ventromedialward and caudad toward the basal plate (cf. Fig. 353-B and C).

In the duck (*Anas platyrhynchos*), the metotic cartilage appears during the eighth day. During the ninth day, it fuses with the lateral edge of the basal plate (Fig. 356-A<sub>1</sub>) behind the vagus nerve (Sonies, 1907), then grows forward, its ventromedial edge fusing with the lateral edge of the basal plate in front of the vagus and its dorsal border fusing to the lateral wall of the pars canalicularis. At the same time, the pars canalicularis grows caudad, and its posterior surface fuses with the anterior edge of the occipital arch. As a result, nothing remains of the fissura metotica by the end of the ninth day except the foramina for the glossopharyngeus and vagus nerves (Fig. 356-B<sub>1</sub> and B<sub>2</sub>) and, anteriorly, the medial aperture of the recessus scalae tympani, the space into which the foramen perilymphaticum opens. The metotic cartilage forms a covering from the side and from behind for the recessus scalae tympani, whose lateral aperture opens into the tympanic cavity. The free edge of the metotic cartilage extends forward and upward almost to the otic process of the quadrate. In the chick,



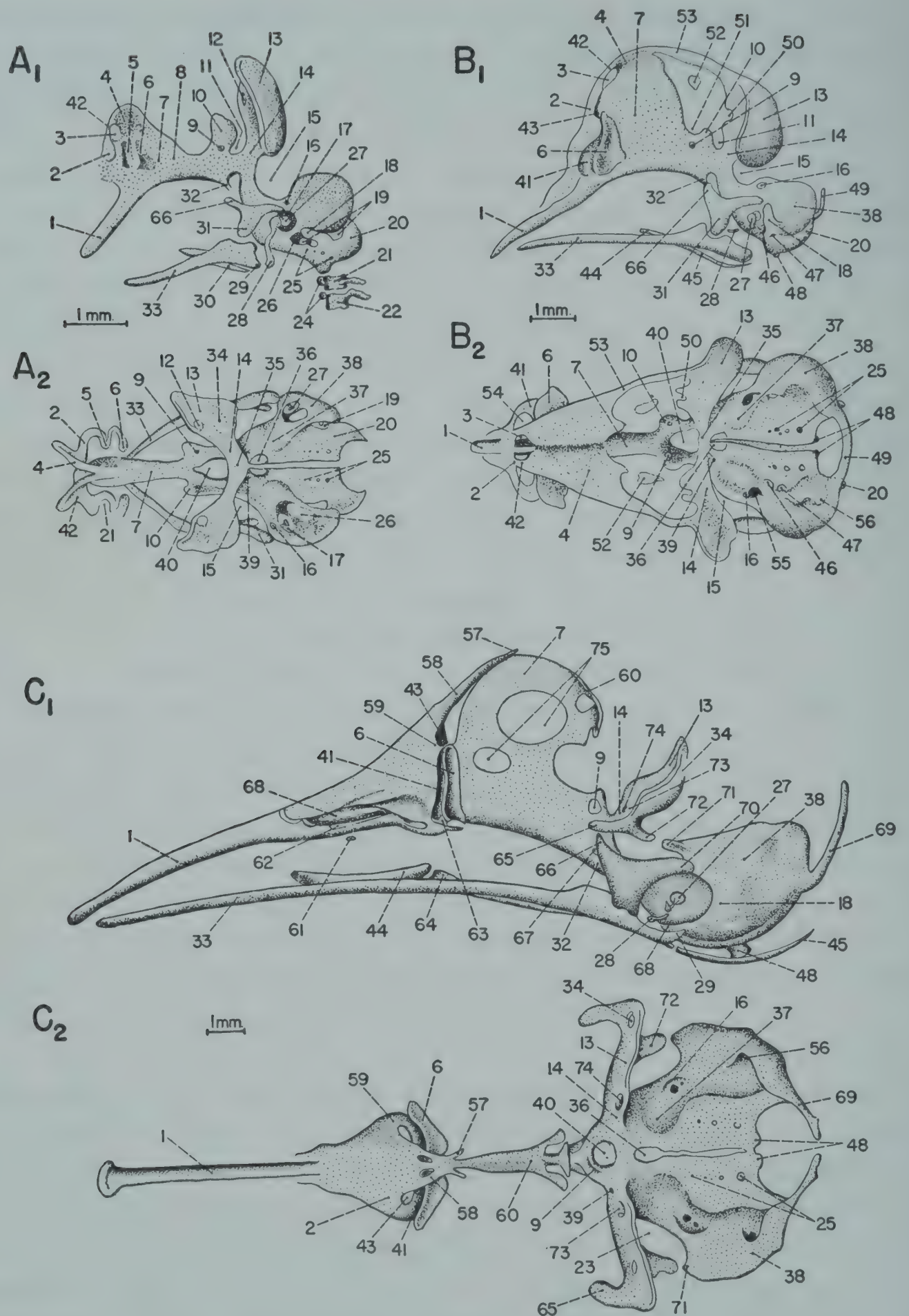


Fig. 356. The chondrocranium of the duck (*Anas platyrhynchos*) at various stages of development between the middle of the ninth day and the end of the fourteenth day of incubation. (Redrawn with modifications after Beer and Barrington, 1934.)

A<sub>1</sub>, A<sub>2</sub>, lateral and dorsal views at 8.5 days of incubation; B<sub>1</sub>, B<sub>2</sub>, same, at 9 days; C<sub>1</sub>, C<sub>2</sub>, same, at 14 days. All in scale.

1, prenasal process; 2, parietotectal cartilage of nasal capsule; 3, foramen olfactorium evehens; 4, anterior portion of orbital cartilage; 5, paranasal cartilage; 6, planum antorbitale; 7, interorbital septum; 8, trabecula communis; 9, foramen for ophthalmic artery; 10, suprapolar cartilage; 11, notch for oculomotor nerve; 12, foramen for trochlear nerve; 13, posterior portion of orbital cartilage; 14, pila antotica; 15, incisura antotica;



this stage is reached by the end of the first week of incubation (*Parker, 1869*). In the 14-day duck embryo, the anterodorsal part of the metotic cartilage has grown forward as the pro-otic process which is almost in contact with the orbitocapsular process of the posterior orbital cartilage (Fig. 356-C<sub>1</sub> and C<sub>2</sub>); and the anteroventral part of the metotic cartilage has formed the subcapsular process (*Beer and Barrington, 1934*), which extends forward as a floor to the recessus scalae tympani.

In the penguin (*Spheniscus demersus*), the metotic cartilage is already present at the 43-mm. stage as a short process extending ventrally from the posterolateral edge of the pars canalicularis. In this species, the metotic cartilage does not participate in the subdivision of the incisura metotica. The vagus foramen is formed by the growth of the basal plate itself (cf. Fig. 355-D), and the glossopharyngeal foramen is formed by the fusion of the basal plate with the pars canalicularis. By the 58-mm. stage, the medial edge of the metotic cartilage has fused with the lateral edge of the basal plate anterolateral to the fusion of the auditory capsule with the basal plate, and the posterior edge of the metotic cartilage has fused with the lateral surface of the auditory capsule (Fig. 353-D). The subcapsular process now develops and fuses to the basal plate. The lateral edge of the subcapsular process forms the ventral border of the fenestra pseudorotunda (so-called because its origin is different from that of the mammalian fenestra rotunda) and the ventral edge of the foramen perilymphaticum forms the dorsal border of the fenestra pseudorotunda, across which stretches the secondary

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16, foramen for facial nerve; 17, foramen ovale of auditory capsule; 18, metotic cartilage; 19, fissura metotica; 20, definitive occipital arch; 21, atlas; 22, axis; 23, foramen pro-oticum spurium (for maxillary and mandibular branches of trigeminal nerve); 24, hypocentra of atlas and axis; 25, foramina for hypoglossal nerve roots; 26, foramen perilymphaticum of auditory capsule; 27, columella auris; 28, stylohyal cartilage; 29, processus retroarticularis of Meckel's cartilage; 30, ceratobranchial of first branchial arch; 31, quadrate; 32, infrapolar process; 33, Meckel's cartilage; 34, vacuity in orbital septum; 35, acrochordal cartilage; 36, basicranial fenestra; 37, cochlear portion of auditory capsule; 38, canalicular portion of auditory capsule; 39, foramen for abducens nerve; 40, hypophyseal fenestra; 41, concha nasalis; 42, sphenethmoid commissure; 43, foramen olfactorium advehens; 44, entoglossal cartilage (ceratohyals fused); 45, epibranchial cartilage; 46, foramen for glossopharyngeal nerve; 47, foramen for vagus nerve; 48, occipital condyle; 49, tectum synoticum; 50, notch for trochlear nerve; 51, notch for optic nerve; 52, rudiment of taenia medialis; 53, supraorbital cartilage; 54, orbitonasal fissure; 55, foramen for auditory nerve; 56, fossa subarcuata; 57, processus tectalis; 58, backgrowing roof of nasal capsule; 59, line of junction between parietotectal and paranasal cartilages; 60, planum suprasedale; 61, prevomer cartilage; 62, fenestra septi nasalis posterior; 63, aperture in nasal capsule communicating with orbital sinus; 64, copula; 65, postorbital process; 66, pterygoid process of quadrate; 67, lateral carotid foramen; 68, subcapsular process; 69, supraoccipital process; 70, otic process of quadrate; 71, pro-otic process; 72, orbitocapsular process; 73, pila antotica spuria; 74, foramen for profundus branch of trigeminal nerve; 75, vacuities in interorbital septum.



tympanic (entotympanic) membrane. No pro-otic process arises from the metotic cartilage in the penguin (*Spheniscus demersus*); as Fig. 357-A<sub>1</sub> shows, the incisura pro-otica is bridged by the orbitocapsular process and thus transformed into a foramen at the 74-mm. stage (Crompton, 1953).

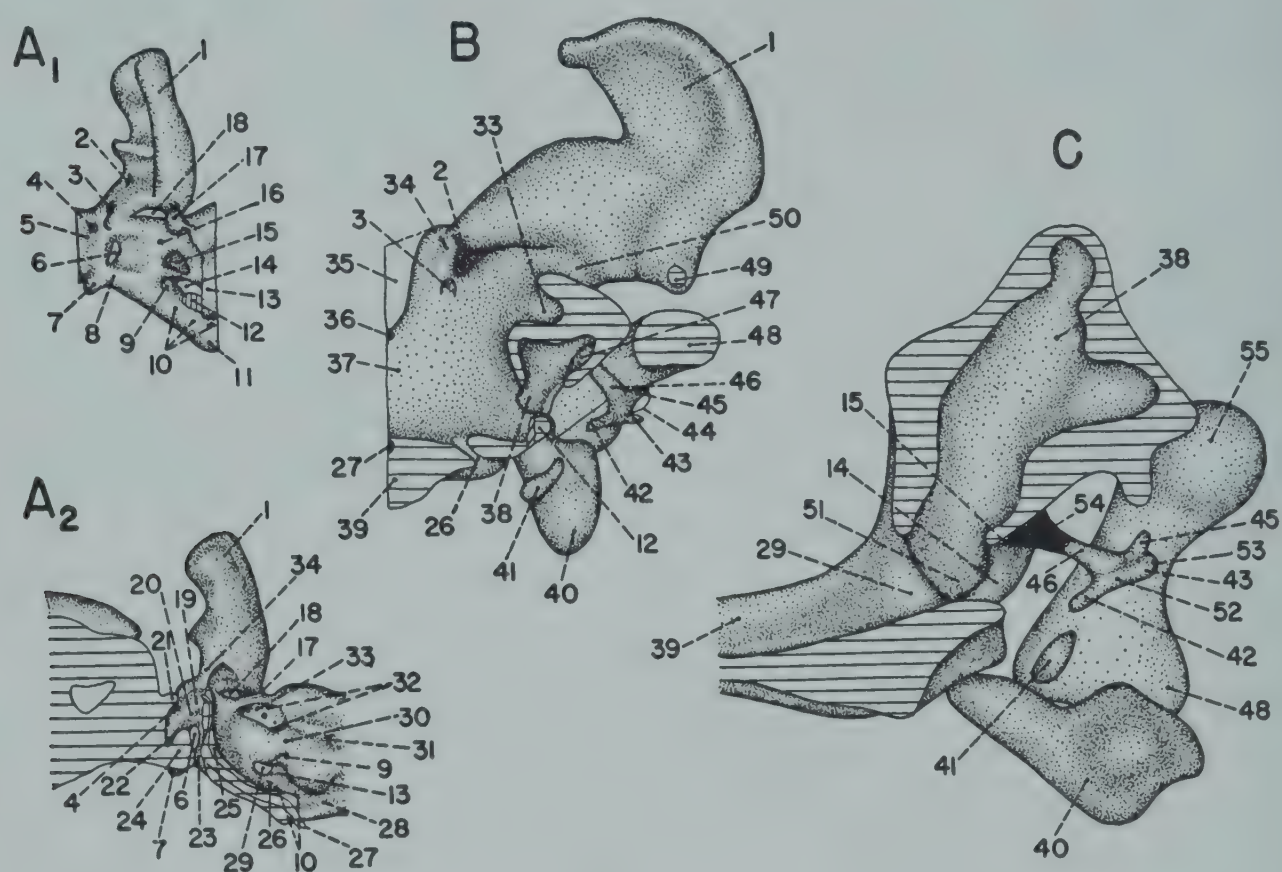


Fig. 357. Stages in the development of the otic capsule and the hypophyseal region in the penguin, *Spheniscus demersus*. (Redrawn with modifications after Crompton, 1953.)

A<sub>1</sub>, left lateral view at 74-mm. stage, after removal of quadrate and metotic cartilages; A<sub>2</sub>, median view after sagittal section, same stage; B, posterior view of right otic capsule at 91-mm. stage after transverse section and removal of more caudal structures; C, right otic capsule at 150-mm. stage, sectioned transversely and viewed from behind. All  $\times 5$ .

1, postorbital cartilage; 2, foramen for the profundus nerve; 3, foramen for the abducent nerve; 4, foramen for the ophthalmic artery; 5, suprapolar cartilage; 6, lateral carotid foramen; 7, basitrabecular process; 8, infrapolar commissure; 9, foramen perilymphaticum; 10, hypoglossal foramen; 11, occipital condyle; 12, section through subcapsular process; 13, metotic cartilage; 14, apertura lateralis recessus scalae tympani; 15, foramen ovale; 16, foramen for the facial nerve; 17, orbitocapsular process; 18, foramen pro-oticum; 19, hypophyseal pit; 20, foramen caroticum; 21, hypophyseal roof; 22, remnant of the foramen hypophyseos; 23, basicranial foramen; 24, precarotid commissure; 25, acrochordal cartilage; 26, foramen for the vagus nerve; 27, notochord; 28, foramen magnum; 29, apertura medialis recessus scalae tympani; 30, dorsal cochleo-canalicular commissure; 31, foramen for ductus endolymphaticus; 32, foramen acusticum; 33, cavity lodging the ganglion geniculatum; 34, pila antotica; 35, membrane; 36, foramen for the tip of the notochord; 37, remnant of the acrochordal cartilage; 38, cavity of the auditory capsule; 39, basal plate; 40, Meckel's cartilage; 41, stylohyal cartilage; 42, processus infracolumellaris; 43, processus extracolumellaris; 44, chorda tympani; 45, processus supracolumellaris; 46, columella rod; 47, footplate of the columella auris; 48, pars quadrata; 49, orbitocapsular process; 50, pila antotica spuria; 51, recessus scalae tympani; 52, extracolumella; 53, foramen in the processus extracolumellaris for the chorda tympani; 54, stapedial ossification; 55, processus oticus.



Other views of the auditory capsule of the penguin at the 74-, 91-, and 150-mm. stages are shown in Fig. 357-A<sub>2</sub>, B, and C.

In the ostrich (*Struthio camelus*), the role of the metotic cartilage in the formation of the glossopharyngeus and vagus foramina is slightly different. In the embryo of 10.5-mm. head length, the metotic cartilage is present and fused with the otic capsule and the basal plate, but the two nerves emerge through a common exit in front of the metotic cartilage. In the embryo of 21-mm. head length, the nerves have separate foramina in the anterior margin of the cartilage (Brock, 1937).

The otic capsules become connected with each other by the tectum synoticum, which forms the dorsal border of the foramen magnum. The tectum synoticum is complete from side to side in the 10- to 11-day chick (Tonkoff, 1900b), the ostrich (*Struthio camelus*) embryo of 10.5-mm. head length (Brock, 1937), the 91-mm. penguin (*Spheniscus demersus*) embryo (Crompton, 1953), and the 9-day duck (*Anas platyrhynchos*) embryo (Fig. 356-B<sub>2</sub>); in the 14-day duck, however, it has become interrupted in the midline (Fig. 356-C<sub>2</sub>), leaving the bilateral supraoccipital processes projecting medialward from the medial dorsoposterior corner of the otic capsule (Beer and Barrington, 1934). The anlage of the tectum synoticum, as seen in the 58-mm. penguin is a continuous blastematous tract connecting the otic capsules; in the kestrel, *Falco tinnunculus* (Suschkin, 1898), the pro-chondral tectum synoticum differentiates *in situ* in continuity with the lateral occipital region and is not proliferated by the latter. The cartilaginous tectum synoticum, however, is said to originate in the chick (Parker, 1869) and the duck (Sonies, 1907) from paired chondrifications whose medial ends grow dorsad and fuse together in the midline before becoming connected with the otic capsule and, slightly later, with the occipital arch. The tectum synoticum, by this account, participates in the closure of the fissura metotica.

**The Orbital Region.** In the chondrocranium, the orbit is composed of the anterior orbital, posterior orbital, and supraorbital cartilages and the interorbital septum. The first of these to begin development is the posterior orbital cartilage, which is located caudal to the eyeball. In the duck (*Anas platyrhynchos*), and probably also in the penguin, *Spheniscus demersus* (Crompton, 1953), it chondrifies in continuity with the pila antotica (Sonies, 1907; Beer and Barrington, 1934). It is first detectable as a dorsal upgrowth of the latter at the end of the duck's seventh day of incubation (cf. Fig. 352-D<sub>1</sub> and D<sub>2</sub>); then enlarges mediolateralward over the posterior surface of the eyeball and, as Fig. 352-E<sub>1</sub> and E<sub>2</sub> shows, has attained considerable size by the middle of the eighth day (Beer and Barrington, 1934). In the chick, the posterior orbital cartilage chondrifies independently at some distance lateral to the pila antotica and spreads in all directions until it finally fuses with the apex of the pila antotica (Sonies, 1907). The



trochlear nerve becomes enclosed in a foramen near the medial edge of the cartilage. In the penguin (*Crompton*, 1953), the trochlear foramen is near the lateral edge at first, though later its position becomes more medial; and the oculomotor nerve is enclosed in a canal located along the original boundary between the pila antotica and the posterior orbital cartilage. This canal forms also in the kiwi, *Apteryx oweni* (*Parker*, 1892a); in the duck, however, the oculomotor nerve emerges through a notch between the pila antotica and the suprapolar cartilage (*Beer and Barrington*, 1934).

The preoptic root of the anterior orbital cartilage appears at the same time or slightly later than the posterior orbital cartilage (cf. Fig. 352-D<sub>1</sub>, D<sub>2</sub>, E<sub>1</sub>, and E<sub>2</sub>). It consists of a curved process projecting laterally, dorsally, and cranially from the anterolateral edge of the trabecula. In the 8-day duck, *Anas platyrhynchos*, the 15-day emu, *Dromaeus novae-hollandiae*, and the 47-mm. penguin, *Spheniscus demersus* (*Beer and Barrington*, 1934; *Lutz*, 1942; *Crompton*, 1953), the interorbital septum is growing up from the middle of the trabecula communis behind the paired preoptic roots, and the anterior orbital cartilage can be recognized as a dorsad extension of the preoptic root (cf. Fig. 352-E<sub>1</sub> and E<sub>2</sub>; Fig. 353-B). In the gull (*Larus* sp.) the anterior orbital cartilage is an independent chondrification (*Beer and Barrington*, 1934).

The orbital cartilages expand rapidly, the posterior cartilage becoming a large, curving structure with concavity presented to the eyeball, and the anterior cartilage spreading out on each side beneath the cerebral hemispheres. The interorbital septum grows upward, carrying the preoptic roots with it. In the 8.5-day duck (*Anas platyrhynchos*), the height of the interorbital septum has increased considerably (Fig. 356-A<sub>1</sub> and A<sub>2</sub>). The anterodorsal corner of the anterior orbital cartilage now becomes attached to the parietotectal cartilage by means of the sphenethmoid commissure (*Beer and Barrington*, 1934).

In the 9-day duck (*Anas platyrhynchos*) embryo, the supraorbital cartilage makes its appearance in the form of a narrow band of cartilage stretching back from the dorsoposterior edge of the anterior orbital cartilage toward the dorsal edge of the posterior orbital cartilage (*Beer and Barrington*, 1934), with which it soon fuses (Fig. 356-B<sub>1</sub>). This connection is established in the 56-mm. penguin, *Spheniscus demersus* (cf. Fig. 353-C); in this species, however, the supraorbital cartilage arises from a rostrally directed process of the posterior orbital cartilage (cf. Fig. 355-C). The interorbital septum is fused dorsally with the anterior orbital cartilage, but a large space intervenes between the posterodorsal edge of the septum and the supraorbital and posterior orbital cartilages (cf. Fig. 356-B<sub>1</sub>). There thus exists on each side a large sphenoid fontanelle, through which emerge the optic, oculomotor, and trochlear nerves, resorption of cartilage having transformed the trochlear foramen into a notch in the duck of this stage.



(Sonies, 1907; Beer and Barrington, 1934); in the penguin, however, the oculomotor and trochlear nerves are not freed until relatively somewhat later stages (Crompton, 1953).

A secondary connection is now established between the posterior orbital cartilage and the otic portion of the basal plate and is accomplished in the 9-day chick and the 12-day duck, *Anas platyrhynchos* (Sonies, 1907), by fusion between the cranially directed pro-otic process of the otic capsule and the caudally directed orbitocapsular process of the posterior orbital cartilage (cf. Fig. 356-C<sub>1</sub> and C<sub>2</sub>). In the penguin (*Spheniscus demersus*), the orbitocapsular process alone forms this connection by the 74-mm. stage (Crompton, 1953). The incisura antotica (the space between the otic capsule and the posterior orbital cartilage) is now transformed into the foramen antoticum, through which the mandibular and maxillary rami of the trigeminus emerge.

The posterior orbital cartilage, in some birds, forms another secondary connection with the basal plate. This connection, known as the pila antotica spuria, joins the posterior orbital cartilage to the basal plate in such a way that the profundus branch of the trigeminus nerve is separated from the maxillary and mandibular rami and enclosed in a foramen between the pila antotica and the pila antotica spuria. The latter may be wider or narrower than the pila antotica and may be behind, lateral to, or in front of it (Kesteven, 1942). In the duck (*Anas platyrhynchos*), the pila antotica spuria is completed between the eleventh and fourteenth days of incubation (Beer and Barrington, 1934) and is situated lateral to the pila antotica; in the ostrich (*Struthio camelus*), it develops while the head length increases from 10.5 mm. to 21 mm. (Brock, 1937), but, as in the emu (*Dromaeus novae-hollandiae*), it remains incomplete (Kesteven, 1942). It appears in the grebe (*Podiceps cristatus*) between the 8.7-mm. and the 11.9-mm. stage, in front of the pila antotica; in the 58-mm. kiwi (*Apteryx oweni*) embryo, and in the stilt (*Himantopus himantopus*), it is found behind the pila antotica (Kesteven, 1942). In the chick, the turkey (*Meleagris gallopavo*), and the zebra parakeet (*Melopsittacus undulatus*), the only connection between the posterior orbital cartilage and the basal plate is lateral to the profundus branch of the trigeminus, in the position of a pila antotica spuria (Kesteven, 1942).

Having attained a maximum size, the cartilages of the orbit begin to undergo reduction. In the 61-mm. penguin (*Spheniscus demersus*), the posterior part of the supraorbital cartilage has atrophied; the ventral portion of the anterior orbital cartilage (that is, the preoptic root) has migrated medially and has become applied against the interorbital septum; and the dorsal portion of the anterior orbital cartilage extends laterally as a horizontal plate, the planum suprasedale (cf. Fig. 358-B). Much the same condition is found in the sparrow (*Passer domesticus*) about to hatch (Beer



and Barrington, 1934) and in the ostrich (*Struthio camelus*) of 10.5-mm. head length, although the preoptic root has atrophied in the latter bird by the time the head length is 21 mm. (Brock, 1937).

In the 74-mm. penguin (*Spheniscus demersus*) embryo, the planum supraseptale has been reduced to a narrow projection (cf. Fig. 358-C<sub>1</sub>), and a large fontanelle has appeared in the interorbital septum (Crompton, 1953). A similar but somewhat more advanced stage is found in the 11-day duck (*Anas platyrhynchos*) embryo (Fig. 356-C<sub>1</sub> and C<sub>2</sub>), in which the sphenethmoid commissure has become interrupted; the planum supraseptale, now only a thin strip, is continuous anteriorly with the posterior remnant of the commissure (the sphenethmoid process). In the 14-day duck (cf. Fig. 356-C<sub>1</sub> and C<sub>2</sub>), the planum supraseptale has regressed still further, and two vacuities are present in the interorbital septum. In the penguin, the regression of the septum continues until only a small anterior portion remains in the adult (Crompton, 1953).

At the beginning of the second week of incubation, cartilage appears in the fibrous sclerotic coat of the eye. At the margin, a series of bony plates develops later (cf. Fig. 161 and 164 in Chapter 5).

**The Posterior Orbitotemporal Region.** The hypophyseal foramen is a space originally bounded by the trabecula communis, the polar cartilages, and either the acrochordal cartilage (Sonies, 1907) or, as in the penguin (*Spheniscus demersus*), the precarotid commissure (Crompton, 1953). As the structures surrounding it fuse together, grow, and change their mutual relationships, the hypophyseal foramen gradually becomes situated at the bottom of a deep pit, the hypophyseal fossa. In the 58-mm. penguin, the anterior border of this fossa is no longer the trabecula communis but the posterior end of the interorbital septum (cf. Fig. 355-D), to whose latero-posterior surfaces the suprapolar cartilages have fused. These cartilages form the lateral walls of the fossa. In this species, the precarotid commissure, which separates the hypophyseal foramen from the median carotid foramen, provides an incomplete ventral wall to the hypophyseal fossa. The lower part of the acrochordal cartilage has migrated forward and is in continuity laterally with the ventromedial part of the basitrabecular process (cf. Fig. 355-D), which has extended medially to lie directly beneath the precarotid commissure; a small basicranial foramen opens forward between the precarotid commissure and the acrochordal. The dorsomedial part of the basitrabecular process is fused to the anteroventral surface of the polar cartilage; the syndesmotic connection between the basitrabecular and the quadrate breaks down by the 61-mm. stage. The changes just described transform the lateral carotid incisure of each side into a foramen (cf. Fig. 355-D), leading, of course, to the median carotid foramen. In birds that possess an infrapolar process, the formation of the lateral carotid foramen is brought about by its fusion with the ventral surface of the basal plate at



a relatively later stage, for example, in the duck (*Anas platyrhynchos*), between the eleventh and fourteenth days of incubation (Beer and Barrington, 1934). The infrapolar process is simulated in the penguin (*Spheniscus demersus*), however, at the 74-mm. stage, when the cranial flexure has straightened to such an extent that the structures constituting the ventral border of the lateral carotid foramen are forced to elongate, forming an infrapolar commissure (cf. Fig. 357-A<sub>1</sub>); a similar condition exists in the 18-mm. ostrich, *Struthio camelus* (Brock, 1937). The virtual disappearance of the flexure also swings the basicranial foramen of the penguin directly below the median carotid foramen (cf. Fig. 357-A<sub>2</sub>).

The cartilages surrounding the hypophyseal fossa are now beginning to atrophy. The medial part of the acrochordal has disintegrated, exposing the anterior (morphologically ventral) surface of the notochord, which protrudes into the basicranial foramen. Much of the dorsal (morphologically anterior) portion of the acrochordal has also atrophied, so that the tip of the notochord projects from it relatively much closer to its dorsal edge (cf. Fig. 357-A<sub>2</sub>). The suprapolar cartilage has begun to atrophy dorsal to the ophthalmic foramen. The trabeculae and the precarotid commissure have fused together in the midline to occlude the hypophyseal foramen. A horizontal prolongation of the interorbital septum projects over the anterior part of the hypophyseal fossa in the form of a roof (cf. Fig. 357-A<sub>2</sub>), above which lies the optic chiasma.

The medial part of the acrochordal has broken down to a membrane in the 91-mm. penguin, *Spheniscus demersus* (cf. Fig. 357-B), so that the notochord projects upward from a medial foramen in the anterodorsal surface of the basal plate; after hatching, a bony lamella forming the posterior wall of the hypophyseal fossa develops in the membranous remnant of the acrochordal (Crompton, 1953). A similar disappearance of the acrochordal has been observed in the kiwi, *Apteryx oweni* (Parker, 1892a), the duck, *Anas platyrhynchos*, the sparrow, *Passer domesticus*, the starling, *Sturnus vulgaris* (Sonies, 1907), and the chick (Tonkoff, 1900b), in which, however, atrophy does not appear to be so extensive as in other birds.

Other changes seen in the 91-mm. penguin (*Spheniscus demersus*) are the closure of the basicranial fenestra by fusion of the infrapolar commissures in the midline, and the disappearance of the suprapolar cartilages. The basitrabecular processes are very reduced in size; although they ossify later, they atrophy after hatching to become ligaments extending between the skull base and the pterygoids (Crompton, 1953).

**The Nasal Capsule.** Development of the nasal capsule can be considered as beginning with the union of the trabeculae in the midline, for the nasal septum, the prenasal process, which is an anterior prolongation of the septum, and the roof of the nasal capsule are derivatives of the trabeculae. In some birds, the lateral walls chondrify in continuity



with the roof, whereas in others they appear to have an independent origin.

In the chick and the duck (*Anas platyrhynchos*), the trabeculae are joined together during the seventh day of incubation. Within a few hours, the first anlage of the nasal septum is seen in the chick as a broad, thick, dorsal outgrowth of the trabecula communis; in the duck, *Anas platyrhynchos* (cf. Fig. 352-D<sub>2</sub>), the nasal septum consists at first of a deepening of the wide ethmoid plate uniting the anterior ends of the trabeculae (Sonies, 1907; Beer and Barrington, 1934). The nasal septum of the kestrel (*Falco tinnunculus*) is said to be derived from the independent intertrabecula (Suschkin, 1898), and that of the penguin (*Spheniscus demersus*) is described as appearing at the 56-mm. stage (cf. Fig. 353-B and C) as an upward extension of the posterodorsal surface of the prenasal process, which is present at the 47-mm. stage (Crompton, 1953). In the chick, the duck (*Anas platyrhynchos*), and the ostrich (*Struthio camelus*), the prenasal process is an outgrowth of the nasal septum (Parker, 1869; Sonies, 1907; Beer and Barrington, 1934; Brock, 1937).

In the beginning, the prenasal process and the nasal septum are inclined downward in such birds as the duck (*Anas platyrhynchos*) and the chick, and particularly in the zebra parakeet (*Melopsittacus undulatus*), which has a curved beak throughout life (cf. Fig. 352-E<sub>1</sub>; Fig. 354-A<sub>2</sub> and B<sub>2</sub>); in later stages, however, the curvature is eliminated or, in the parakeet, reduced (cf. Fig. 354-A). In the pigeon (*Columba livia*), however, the declination of the ethmoidal region is not present (cf. Fig. 354-C).

In the 56-mm. penguin, *Spheniscus demersus* (Crompton, 1953), the 17-mm. chick, and the 8-day duck, *Anas platyrhynchos* (Sonies, 1907; Beer and Barrington, 1934), the dorsal edge of the nasal septum is beginning to extend to each side to form the parietotectal cartilages (cf. Fig. 352-E<sub>1</sub> and E<sub>2</sub>; Fig. 353-C). These will give rise to the roof of the nasal capsule (the tectum nasi).

At the same time (or, in the duck, *Anas platyrhynchos*, at the 8.5-day stage), the sphenethmoid commissure appears and connects the parietotectal cartilage with the anterior orbital cartilage on each side (cf. Fig. 353-C; Fig. 356-A). The sphenethmoid commissure forms the lateral border of the foramen olfactorium evehens, whose remaining borders consist of the preoptic root and the anterior orbital cartilage, the nasal septum, and part of the posterior edge, of the parietotectal cartilage. The olfactory nerve passes down through this foramen and (except in the penguin, *Spheniscus demersus*) runs for a short distance in the orbit, beneath the parietotectal cartilage, before entering the nasal cavity (Beer and Barrington, 1934). In the penguin, however, the foramen olfactorium evehens opens directly into the nasal capsule, because the preoptic root does not grow high enough to carry the foramen dorsalward (Crompton, 1953).



Also at this stage, the lateral wall of the nasal capsule begins to develop. In some birds, including the penguin, *Spheniscus demersus* (Crompton, 1953), the kestrel, *Falco tinnunculus* (Suschkin, 1898), the ostrich, *Struthio camelus* (Brock, 1937), and perhaps the chick (Born, 1879), the lateral wall is established in continuity with the roof. In the duck, *Anas platyrhynchos* (Sonies, 1907; Beer and Barrington, 1934), and probably in the chick (Sonies, 1907), the formation of the lateral wall begins with the appearance of the paranasal cartilage as an independent chondrification (cf. Fig. 356-A<sub>1</sub> and A<sub>2</sub>). This cartilage, which supports the superior concha (see Chapter 5), forms at a short distance lateral to the nasal septum. As seen from the dorsal surface, it is a U-shaped element with concavity directed lateralward. It soon fuses with the parietotectal cartilage. In the penguin (*Spheniscus demersus*), the entire lateral wall of the nasal capsule exists at the 58-mm. stage as a precartilaginous plate growing down from the parietotectal cartilage, lateral to the nasal sac (Fig. 353-D). As in the kestrel (*Falco tinnunculus*), the portion of the lateral wall that is to support the superior concha shows no curvature in the beginning; in the 61-mm. penguin embryo, it starts to fold into the cavity of the capsule in a medial and slightly anterior direction (Crompton, 1953).

The posterior wall of the nasal capsule is formed by the planum antorbitale, which, in most birds, is a vertical plate of cartilage placed almost at a right angle to the nasal septum. In the duck (*Anas platyrhynchos*), the planum antorbitale appears at the same time as the paranasal cartilage and behind it (cf. Fig. 356-A<sub>1</sub> and A<sub>2</sub>), and likewise an independent chondrification (Beer and Barrington, 1934); in the chick, it is established somewhat later than, and in continuity with, the paranasal (Sonies, 1907). In the kestrel, *Falco tinnunculus* (Suschkin, 1898), the upper part of the planum antorbitale arises as a process of the posterior edge of the paranasal; the lower part consists of a lateral continuation of the trabecula and is connected with the interorbital septum by connective tissue. In the 58-mm. penguin, *Spheniscus demersus* (cf. Fig. 353-D), the planum antorbitale is represented by a small transverse plate proceeding from the lower posterior part of the lateral wall of the nasal capsule.

The planum antorbitale forms one of the borders of the orbitonasal fissure, a space providing communication between the cavity of the nasal capsule and the orbit (Fig. 358-A and B). In the duck (*Anas platyrhynchos*), the orbitonasal fissure is bound dorsally by the sphenethmoid commissure, ventrally by the planum antorbitale, medially by the interorbital septum and the preoptic root, and laterally by part of the posterior edge of the parietotectal cartilage (Beer and Barrington, 1934). The medial nasal ramus of the profundus nerve enters the nasal capsule through the upper part of the orbitonasal fissure. In the 58-mm. penguin (*Spheniscus demersus*), a broad plate of cartilage, the postprofundal commissure, has



grown upward from the dorsal edge of the planum antorbitale, medial to the medial ramus, and has fused with the sphenethmoid commissure, thus enclosing the medial ramus in a foramen (cf. Fig. 353-D).

The prenasal process grows far forward, and the tectum nasi extends itself both anteriorly and posteriorly. In the penguin (*Spheniscus demersus*) the postprofundal commissure also grows back to become a wide plate close to the nasal septum, first narrowing the dorsal part of the orbitonasal fissure (Fig. 358-C<sub>1</sub>) and then obliterating it by fusing with the septum at the 91-mm. stage. The excessive development of the postprofundal commissure

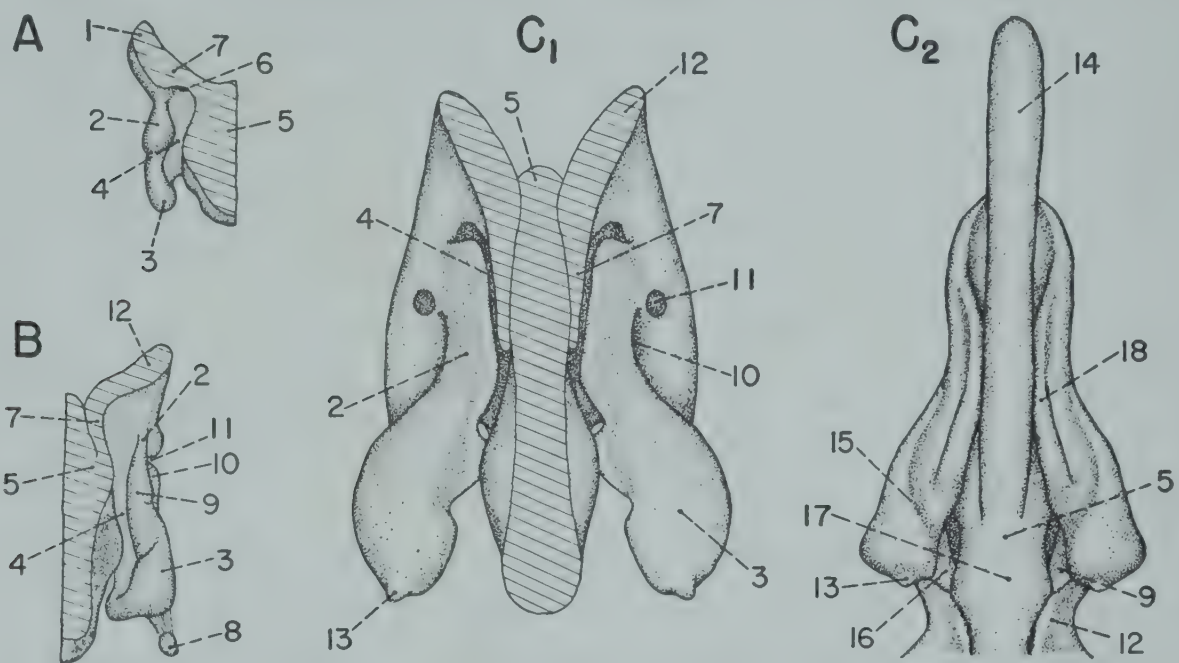


Fig. 358. The developing nasal capsule of the penguin embryo, *Spheniscus demersus*. (Redrawn with modifications after Crompton, 1953.)

A, reconstruction of a portion of the hinder region of the nasal capsule, from 58-mm. stage; B, same, from 61-mm. stage; C<sub>1</sub>, posterior view of reconstruction of the nasal capsule from 74-mm. stage; C<sub>2</sub>, ventral view of reconstruction of the nasal capsule from 74-mm. stage. All  $\times 8$ .

1, anterior orbital cartilage; 2, postprofundal commissure; 3, pila antotica; 4, orbito-nasale fissure; 5, nasal septum; 6, foramen olfactorium evehens; 7, preoptic root of the orbital cartilage; 8, cartilage uncinata; 9, concha nasalis; 10, aditus concha; 11, foramen of the ramus medialis nasi; 12, planum suprasedale; 13, os uncinatum; 14, prenasal process; 15, rudimentary floor of the nasal capsule; 16, aperture in the nasal capsule communicating with the orbital sinus; 17, interorbital septum; 18, maxilloturbinal.

occurs at the expense of the planum antorbitale, which remains rudimentary. In the 61-mm. embryo, the posteroventral edge of the planum antorbitale has turned inward to form a rudimentary floor (Fig. 358-B). At the 74-mm. stage, the supporting cartilage of the inferior concha has appeared, growing inward from the lower part of the lateral wall of the capsule below the superior concha; it is a long, scroll-like structure, or half-cylinder, with concavity facing laterally and downward (Fig. 358-C<sub>2</sub>). Posteriorly, it forms an anterior continuation of the rudimentary floor provided by the planum antorbitale. The nasal septum, although low anteriorly, is as high posteriorly as the interorbital septum and contains a small oval



posterior fenestra. The walls of the cartilaginous superior concha are in contact with each other at the anteroventral end, that is, the fold is beginning to collapse to form a solid rather than a hollow structure (Crompton, 1953).

A somewhat more advanced stage of development is seen in the 14-day duck (*Anas platyrhynchos*) embryo, in which—as in the 10-day chick (Sonies, 1907)—the nasal septum contains two fenestrae (Beer and Barrington, 1934). The tectum nasi has grown back to fuse with the interorbital septum immediately in front of the tectal process (cf. Fig. 356-C<sub>1</sub>), which is the anterior remnant of the sphenethmoid commissure, now interrupted. The tectal process is very long in the 10- to 11-day chick embryo (Tonkoff, 1900b). Because of the disappearance of the anterior orbital cartilage (planum suprasedale), which formerly separated the olfactory nerve from the orbit, this nerve, in the duck (*Anas platyrhynchos*), now courses forward within the orbit and beneath the most posterior part of the tectum nasi (Beer and Barrington, 1934). In the sparrow, *Passer domesticus* (Beer and Barrington, 1934), and the penguin, *Spheniscus demersus* (Crompton, 1953), however, the olfactory nerve is enclosed in a canal between the preoptic root and the interorbital septum when the former becomes applied against the latter. The same situation exists in the ostrich (*Struthio camelus*) embryo of 10.5-mm. head length, but the olfactory nerve once more lies within the orbit when it is exposed by the atrophy of the anterior orbital cartilage in the embryo of 21-mm. head length (Brock, 1937).

In the 14-day duck (*Anas platyrhynchos*) embryo, the planum antorbitale is well developed and is still a large plate situated immediately behind the superior concha. Ventral to its lateroventral edge and to the aperture of the superior concha, and dorsal to the floor formed by the posterior end of the inferior concha, is the aperture placing the nasal sac in communication with the orbital sinus.

In the 10- to 11-day chick (Tonkoff, 1900b), the 17-day duck, *Anas platyrhynchos* (Beer and Barrington, 1934), and the 91-mm. penguin, *Spheniscus demersus* (Crompton, 1953), the anterior part of the nasal capsule (the vestibule or atrium) is chondrified. The copula anterior, which bounds the fenestra narina anteriorly, has developed on each side from the parietotectal cartilage, which forms a narrow roof and has grown downward as an incomplete lateral wall. In the duck and the kestrel (*Falco tinnunculus*), but not in the chick (Gaupp, 1905) or the penguin, there is a floor to the anterior part of the vestibule. The cartilaginous vestibular concha is present as a plate extending downward from the anterior part of the parietotectal cartilage; the posterior part of the vestibular concha is lateral to the inferior concha. The latter exhibits one and a half turns in the 17-day duck (Beer and Barrington, 1934) and two full turns in the 6-day hooded



crow, *Corvus cornix* (Parker, 1872) and the 14-day chick (Parker, 1869), but no more than about three quarters of a turn in the penguin. In the 91-mm. penguin embryo, the lateral walls of the superior conchae are completely fused with each other. All three conchae are present in the ostrich (*Struthio camelus*) of 21-mm. head length; the inferior concha exhibits a bifurcation (Parker, 1866; Brock, 1937), which has also been seen in the 17-day embryo of the zebra parakeet, *Melopsittacus undulatus* (Dieulaufé, 1905). Three defects exist in the nasal septum, an anterior and a posterior fenestra and, posterior to the latter, the craniofacial foramen, whose presence makes possible the movement of the upper beak.

In the penguin (*Spheniscus demersus*), the mesethmoid region of the nasal septum (a broad, thick region situated behind the posterior fenestra and dorsal to the craniofacial foramen) ossifies soon after hatching, as does the postprofundal commissure and the planum antorbitale; the anterior part of the nasal septum remains cartilaginous. In the chick, the prenasal process regresses late in the incubation period, as does the lower part of the nasal septum in the vestibular region (Parker, 1869; Born, 1879); in the kestrel (*Falco tinnunculus*), most of the nasal capsule ossifies, including the prenasal process (Suschkin, 1898).

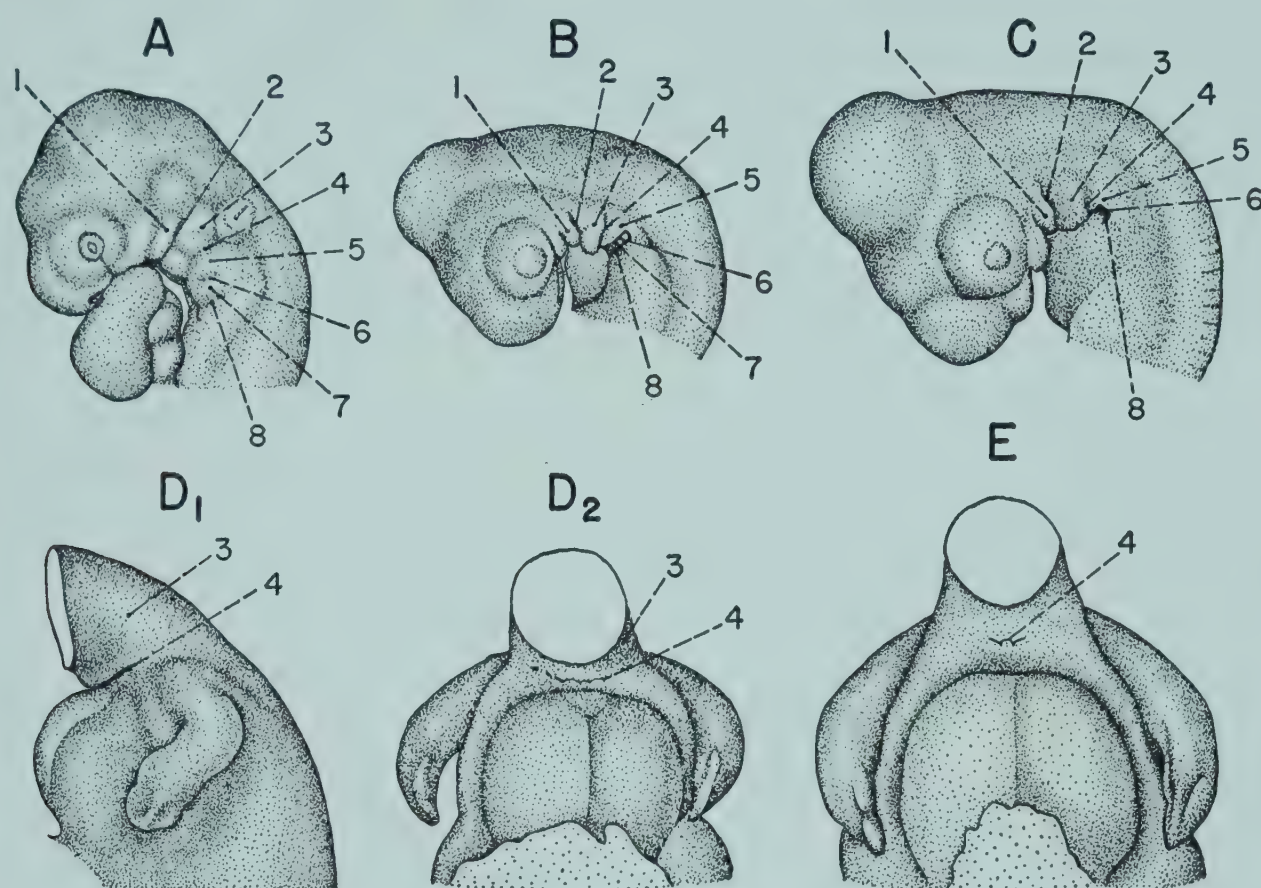
### *The Visceral Arch Skeleton*

Included in the chondrocranium are cartilages derived from the visceral arches. The latter, with their associated clefts, furrows, and pouches (see Chapter 6), are vestiges of the branchial or gill arches and apertures of primitive ancestral forms. Reduction in the number of gill arches and modification of their function is characteristic of the evolutionary process. Even in elasmobranchs, the first arch (mandibular) is transformed into jaws, and only part of the second (hyoid) serves as a gill arch. In land animals, including birds, the visceral arches are present only during embryonic development, and it is difficult to trace the homologies between the persisting visceral skeletal elements and the cartilages that support the branchial arches in elasmobranchs. These cartilages, from dorsal to ventral, are the pharyngo-, epi-, cerato-, and hypobranchial on each side; a basi-branchial or copula lies in the midline between the hypobranchials.

The visceral arches appear in the avian embryo as paired mesodermal thickenings of the lateral and ventrolateral walls of the pharyngeal region. Not only do they form a series of transverse ridges on the outer surface of the body, they also bulge into the pharyngeal cavity. Only four pairs are recognizable externally; two more extremely vestigial pairs are perhaps present, but only as internal protuberances. Each of the two anterior pairs becomes a complete arch by the ventrad growth and median fusion of its lateral halves; the third pair loses its identity by fusing with the second arch, and the fourth regresses. The skeletal elements of the first visceral arch



will support the lower jaw; those of the second arch will provide the columella auris of the ear and part of the hypobranchial skeleton, or supporting framework of the tongue; and those of the third visceral (first branchial) arch contribute the remainder of the hypobranchial skeleton. The skeleton of the fourth visceral (second branchial) arch is represented by a transitory blastema that disappears early in embryonic life. The vestigial arches make no skeletal contributions.



**Fig. 359.** The visceral arches of the chick embryo as seen from the exterior at various times from the second to the seventh day of incubation. (Redrawn with modifications after Moldenhauer, 1877; Boyden, 1918.)

A, lateral view of embryo at 2 days; B, the same, at 3 days; C, the same, at 5 days; D<sub>1</sub>, D<sub>2</sub>, lateral and front views of 6-day embryo; E, front view of chick at 7 days. All  $\times 4$ .

1, first visceral (mandibular) arch; 2, first visceral furrow or cleft; 3, second visceral (hyoid) arch; 4, second visceral furrow, or cleft; 5, third visceral (first branchial) arch; 6, third visceral furrow; 7, fourth visceral (second branchial) arch; 8, fourth visceral furrow.

In the chick, three or four pairs of visceral arches have formed by the end of the second day of incubation (*Reichert, 1837; Moldenhauer, 1877; Kastschenko, 1887; Mall, 1887*); see Fig. 359-A. At the end of the third day (Fig. 359-B), the four arches are wedge-shaped, with broad bases and narrow distal extremities; because of the curvature of the body, their axes form the radii of a circle whose center lies approximately opposite the apex of the second arch. The maxillary process, behind the eye, overlaps the cranial edge of the first arch. The second arch is the largest; it is about twice the size of the third arch, which in turn is larger than the fourth. Twelve to twenty-four hours later (*Mall, 1887; Boyden, 1918*), the first and second arches are massively developed as compared with the third and



fourth, and the distal (ventral) ends of their respective lateral halves are very close to each other. This condition is reached by the duck embryo between the 36- and 42-somite stages (*Kallius, 1905*). A pair of projections on the inner wall of the pharynx, between the rudimentary fourth and fifth visceral pouches, probably represents the fifth pair; these appear in the duck (*Anas platyrhynchos*) embryo before the middle of the fifth day of incubation (*Rabl, 1907b*). It is at about this time that the visceral arches are most clearly seen (*Kastschenko, 1887*). Late in the chick's fourth day or the duck's fifth, the visceral arches begin to lose their regularity because of the marked development of the first and second arches and the reduction of the third and fourth. The posterior edge of the second arch begins to overlap the third. A vestigial sixth arch, even smaller than the fifth, appears medial to the latter. By the end of the chick's fifth day, the first arch has grown much larger, and its halves have fused together in the midline to form the undivided primordium of the lower jaw. The dorsal part of the posterior margin of the second arch has grown backward sufficiently far to cover most of the third arch (Fig. 359-C) and has thus formed a structure homologous to the operculum or gill cover of fishes (*Boyden, 1918*). This structure is said to be especially well developed in embryos of the kiwi, *Apteryx australis* (*Parker, 1890, 1892a*), and the ostrich, *Struthio camelus* (*Nassonow, 1895*). The third and fourth arches are fusing together and flattening out. During the first half of the chick's sixth day, the hyoid arch continues to grow backward, and its two halves meet and fuse in the ventral midline. The underside of the opercular fold now fuses with the surface of the neck from the sides toward the midline (Fig. 359-D<sub>1</sub> and D<sub>2</sub>). Simultaneously, the neck increases in thickness and the visceral arches bulge less prominently into the pharyngeal cavity, so that the wall of the pharynx finally becomes smooth. By the end of the chick's seventh day, all that remains of the hyoid arch externally is a median ventral protuberance (Fig. 359-E). This in turn disappears by the end of the eighth day (*Mall, 1887; Boyden, 1918*).

**The First Visceral Arch—the Quadrate and Meckel's Cartilage.** On each side, the first visceral arch provides an element, Meckel's cartilage, which acts more or less as a foundation for the formation of the membrane bones of the definitive lower jaw. The jaw articulates with the quadrate bone, also derived from the first visceral arch, and the quadrate in turn articulates with the quadratojugal and pterygoid bones, and with either the squamosal or pro-otic bone, or both. In the embryo, the quadrate is the most dorsal element found in the first visceral arch, although it is possible that the spheroidal anlage of the basitrabecular process, which is found above and syndesmotically connected to the quadrate in the 43-mm. penguin, *Spheniscus demersus*, is a vestige of a pharyngomandibular (*Crompton, 1953*).



According to Kallius (1905), Meckel's cartilage and the quadrate, in both the duck, *Anas platyrhynchos*, and the sparrow, *Passer domesticus*, are continuous with each other while still in the precartilaginous state. Crompton (1953) noted a syndesmotic connection between the two cartilages in the penguin (*Spheniscus demersus*) embryo of the earliest stage (43-mm.) that he studied.

The quadrate, which chondrifies before Meckel's cartilage, becomes recognizable in the chick or duck (*Anas platyrhynchos*) embryo during or at the end of the sixth day (Sonies, 1907; Beer and Barrington, 1934). At the time of its appearance, the quadrate is a spheroidal nodule in the chick (Sonies, 1907), but in the duck it is a slender, curved element whose anterior end is directed slightly medialward (cf. Fig. 352-C<sub>1</sub> and C<sub>2</sub>). It is somewhat triangular in the ostrich (*Struthio camelus*) embryo, at a time when Meckel's cartilage is merely a mesenchymatous thickening (Brock, 1937).

The quadrate now begins to send out the otic process from its posterodorsal corner and the orbital or pterygoid process from its anterodorsal corner; most of the latter is blastematous in the 43-mm. jackass penguin, *Spheniscus demersus* (Crompton, 1953). Because of the further development of these processes and the ventral prolongation of its ventral articular process (which articulates with the posterior end of Meckel's cartilage), the quadrate is T-shaped in the 6.5-day chick, the 7.5-day duck (*Anas platyrhynchos*), the 11-mm. starling (*Sturnus vulgaris*), the 15-day emu (*Dromaeus novae-hollandiae*), and the 56-mm. penguin (Sonies, 1907; Beer and Barrington, 1934; Lutz, 1942; Crompton, 1953). In the chick (Parker, 1869) and penguin (Crompton, 1953) of this stage, the otic process has reached and articulates with the otic capsule and the anterodorsal surface of the metotic cartilage (cf. Fig. 353-C). The articulation of the otic process with the skull is present in the 8.5-mm. sparrow (*Passer domesticus*) embryo, in which the orbital process is considerably longer than the otic process (Kallius, 1905). In the other species mentioned above the otic process subsequently grows more rapidly than the orbital process and becomes the larger of the two. In the duck (*Anas platyrhynchos*), the otic process does not effect its articulation with the pro-otic process (formed by the forward growth of the metotic cartilage) until some time between the ninth and fourteenth days of incubation (Beer and Barrington, 1934); the pro-otic process never develops in the penguin, *Spheniscus demersus* (Crompton, 1953). In the ostrich (*Struthio camelus*) of 21-mm. head length, the quadrate articulates with the metotic cartilage and a small parotic process of the otic capsule (Brock, 1937). In the 9- or 10-day chick embryo, the otic process articulates medially with the pro-otic process and laterally with the medial part of the ventral surface of the squamosal bone which is a membrane bone (Sonies, 1907). The articulation of the quadrate



with the posterior end of the quadratojugal, also a membrane bone, occurs later; it is seen in the 58-mm. penguin (cf. Fig. 353-D), the 23.5-mm. sparrow, and the duck incubated more than 2 weeks (*Kallius*, 1905).

Meckel's cartilage is first seen during the chick's sixth day of development (*Sonies*, 1907) and between the middle and end of the duck's (*Anas platyrhynchos*) seventh day (*Sonies*, 1907; *Beer and Barrington*, 1934). In the latter bird, it is derived from two chondrifications (cf. Fig. 352-D<sub>1</sub>), a proximal (posterodorsal) and a distal (anteroventral), of which the proximal one appears first. These have united in the 7.5-day duck (*Beer and Barrington*, 1934) to form a slender, elongated element (cf. Fig. 352-E<sub>1</sub> and E<sub>2</sub>), whose posterior end is thick and whose anterior end curves medialward (*Kallius*, 1905; *Sonies*, 1907). In the chick, the earliest rudiment of Meckel's cartilage is rod-shaped. *Sonies* (1907) was unable to detect any evidence that it has a double origin in either the chick or the starling, *Sturnus vulgaris*. The rod-shaped structure of the chick very soon becomes thick posteriorly, as it already has in the 43-mm. embryo of the penguin, *Spheniscus demersus* (*Crompton*, 1953). In the duck (*Anas platyrhynchos*), the posterior enlargement is chiefly on the lateral surface, where a lateral process becomes recognizable (*Kallius*, 1905). Subsequently, additional processes appear: the retroarticular posteriorly and the medial angular medially; these form during the chick's and duck's eighth and ninth incubation days (*Kallius*, 1905; *Sonies*, 1907; *Beer and Barrington*, 1934) and are present in the 11-mm. sparrow (*Passer domesticus*) embryo (*Kallius*, 1905). The articular processes are less pronounced in the ostrich, *Struthio camelus* (*Parker*, 1866), and the emu, *Dromaeus novae-hollandiae* (*Lutz*, 1942), than in carinate birds. Later (approximately by the duck's fourteenth day of development) a crest forms in the middle of the articular surface; this fits into a groove in the articular surface of the quadrate (*Sonies*, 1907).

The shaft of Meckel's cartilage soon becomes S-shaped as the distal ends align themselves parallel with each other in the tip of the lower jaw. An additional medialward convexity may form at the proximal end of the cartilage and is especially well seen in the embryo of the yellowhammer, *Emberiza citrinella*. These curvatures of Meckel's cartilage characterize sauropsidian embryos (*Lebedinsky*, 1916). The most outstanding phenomenon in the development of Meckel's cartilage is the rostral elongation of its shaft. About twice as long as the quadrate during the duck's (*Anas platyrhynchos*) eighth day, it is nearly four times as long by the end of the ninth (*Kallius*, 1905; *Beer and Barrington*, 1934); thereafter, it keeps pace with the prenasal process and is about six times as long as the quadrate on the fourteenth day (cf. Fig. 352-E<sub>1</sub> and E<sub>2</sub>; Fig. 356-B<sub>1</sub> and C<sub>1</sub>). (Later, the growth of the quadrate reduces this ratio once more to 4:1.) As Meckel's cartilage lengthens, it gradually loses its curvature and



is a thin, straight rod in the 14-day duck and the 23.5-mm. sparrow, *Passer domesticus* (Kallius, 1905; Sonies, 1907). Its anterior end is extremely close to that of the corresponding cartilage of the opposite side, but fusion does not occur. At this stage, membrane bones are already investing the shaft of Meckel's cartilage.

**The Second Visceral Arch—the Columella Auris.** The second or hyoid arch is composed of a median ventral element and paired lateral elements. The median ventral element appears first but joins with the cartilages of the third visceral (first branchial) arch to become part of the hyobranchial skeleton. The lateral elements of each side give rise to the columella auris of the middle ear and to the stylohyal cartilage, which, in some birds, is in cartilaginous continuity with the columella. The columella is sometimes regarded as consisting of a proximal bony portion, the stapes or otostapes, and a distal cartilaginous portion, the hyostapes or extracolumella. The medial end of the columella is enlarged to form the footplate, which rests against the foramen ovale. The distal end gives off three processes, which are in intimate contact with the tympanic membrane.

Accounts describing the development of the skeletal elements of the hyoid arch are at considerable variance. A large measure of clarity, however, was provided by Crompton (1953), who detected four blastemas in each half of the hyoid arch of the 43-mm. penguin (*Spheniscus demersus*) embryo and designated them, from dorsal to ventral, as the pharyngohyal, epihyal, ceratohyal, and hypohyal (Fig. 360-A; cf. Fig. 353-A). The pharyngohyal corresponds to the otostapes. At this early stage, it is connected syndesmotically with the ventromedial surface of the pars canalicularis of the otic capsule; a procartilaginous continuity between these two parts has also been seen in the 6-day chick embryo (Smith, 1904). The epihyal, which corresponds to the hyostapes, is joined by a tract of connective tissue to the ventral edge of the pharyngohyal. The hypohyal, which will give rise to the stylohyal, is a long rod-shaped structure connected with copula I, or the anterior portion of the median ventral element of the hyobranchial skeleton.

While still blastematous, the hypohyal loses its connection with copula I and fuses with the ceratohyal to form a long, curved structure (Fig. 360-B; cf. Fig. 353-B). The pharyngohyal grows anteromedially to form a short blunt process, from which will be derived the major part of the columella rod. The lateral end of the epihyal grows dorsally.

In the 56-mm. penguin, *Spheniscus demersus* (Fig. 360-D), the pharyngohyal and epihyal have fused together and have chondrified to form the columella auris, which is now in cartilaginous continuity with the auditory capsule; this continuity has also been seen in the ostrich, *Struthio camelus* (Brock, 1937), but has not been found in the emu, *Dromaeus novae-hollandiae* (Lutz, 1942). The dorsal extension of the epihyal has chondrified



as the supracolumellar process; in the penguin, this process corresponds only to the lateral portion of the supracolumellar process of other birds, or is equivalent to the laterohyal process. (The medial portion of the supracolumellar process never appears in the penguin.) The extracolumellar process has developed from the base of the epihyal. It is directed antero-

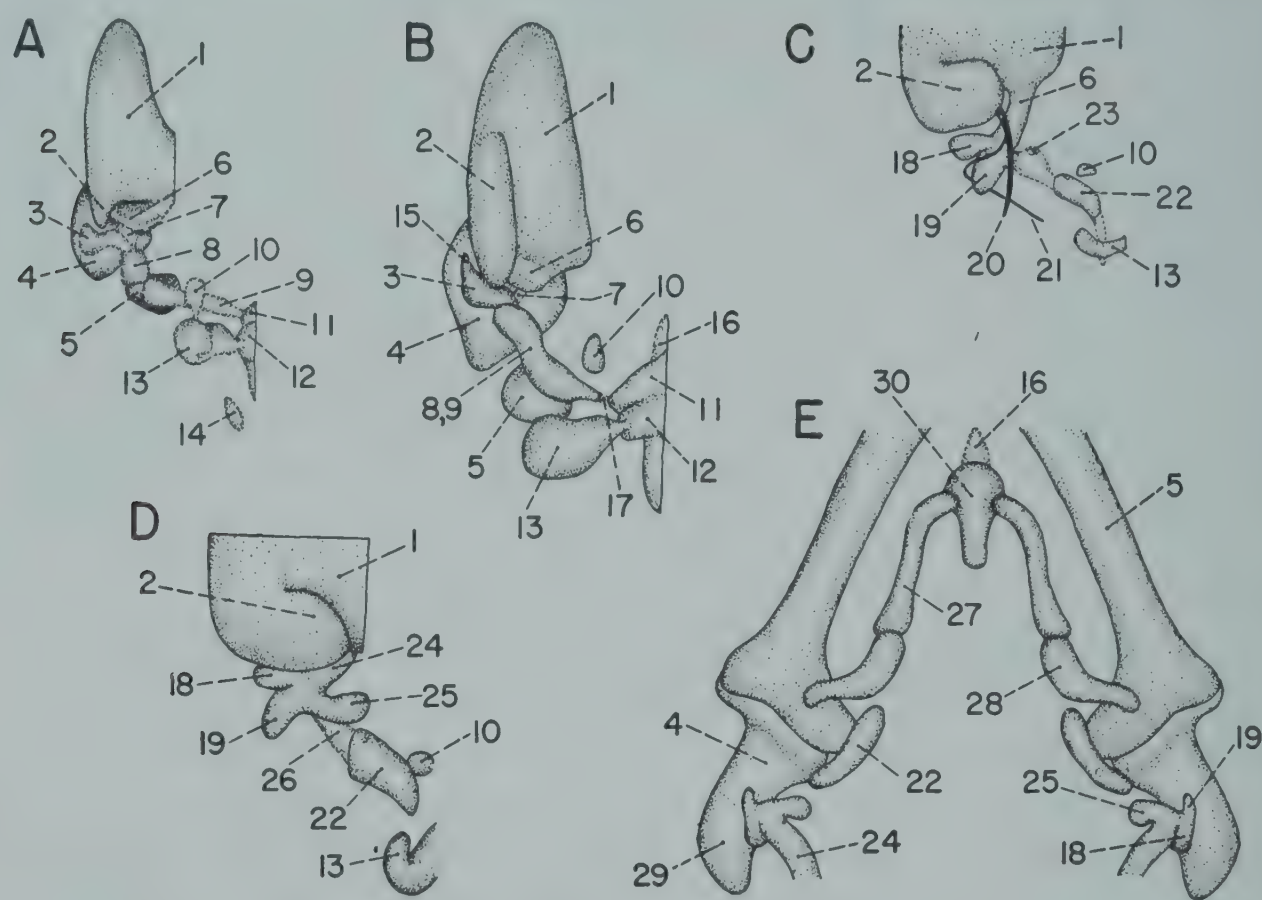


Fig. 360. Development of the cartilages of the second (hyoid) visceral arch in the penguin embryo, *Spheniscus demersus*. (Redrawn with modifications after Crompton, 1953.)

A, posterior view of reconstruction of second visceral (hyoid) arch from 43-mm. stage ( $\times 15$ ); B, the same, from 47-mm. stage ( $\times 15$ ); C, the same, from 51-mm. stage ( $\times 15$ ); D, the same, from 56-mm. stage ( $\times 15$ ); E, ventral view of second visceral arch from 61-mm. stage ( $\times 10$ ).

1, pars canalicularis; 2, metotic cartilage; 3, epihyal; 4, pars quadrata; 5, Meckel's cartilage; 6, pharyngohyal; 7, connective tissue band; 8, ceratohyal; 9, hypohyal; 10, pharyngobranchial; 11, first copula; 12, second copula; 13, first branchial arch; 14, second branchial arch; 15, processus supracolumellaris lateralis; 16, processus lingualis; 17, blastematos connection between the hyoid arch and the first copula; 18, processus supracolumellaris; 19, processus extracolumellaris; 20, hyomandibular nerve; 21, chorda tympani; 22, stylohyal cartilage; 23, ceratohyal chondrification; 24, columella rod; 25, processus infracolumellaris; 26, blastematos connection between the columella auris and the stylohyal cartilage; 27, ceratobranchial; 28, epibranchial; 29, processus oticus; 30, copula.

ventrally and slightly laterally and is cartilaginous. The stylohyal is chondrifying in the ventral part of the ceratohypohyal complex, medioventral to the columella auris, and a small nodule of cartilage is present in the dorsal (ceratohyal) portion. The ceratohyal chondrification soon fuses to the columella auris, but its medial portion grows medially to form the infra-



columellar process. This process does not develop in the starling (*Sturnus vulgaris*). The unchondrified remainder of the ceratohyal connects the stylohyal and the columella auris for some time, but has atrophied by the 74-mm. stage (cf. Fig. 357-B and C). At this time, all three processes of the columella auris are embedded in the tympanic membrane. The foramen ovalis has formed through the atrophy of the cartilage of the pars canalicularis of the otic capsule adjacent to the medial end of the columella.

The development of the columella auris proceeds somewhat differently in the duck (*Anas platyrhynchos*). Crompton (1953) observed precartilaginous centers, corresponding to the pharyngohyal, epihyal, and hypohyal, in the 4.5-day duck embryo. As in most birds, no ceratohyal chondrifies in this species, but a precartilaginous tract, the interhyal, connects the epihyal and hypohyal at the 4.5-day stage. A small arch (the "supracolumellar arcade") of dense blastematos tissue containing a nodule of cartilage extends from the dorsolateral end of the pharyngohyal to the dorsolateral end of the epihyal, enclosing a small space, the foramen of Huxley. Suschkin (1898), who observed the arcade and nodule in the kestrel (*Falco tinnunculus*), believed that the chondrification represented the vestigial skeleton of the operculum. In the 5.5-day duck embryo, the pharyngohyal has chondrified medially to form the footplate of the columella auris. According to Beer and Barrington (1934) and Lutz (1942), there is no cartilaginous continuity between the columella and the otic capsule in the duck. The axes of the pharyngohyal and epihyal now form a right angle, open medially (Kallius, 1905). The supracolumellar chondrification is larger. In the 6.5-day embryo, the foramen ovalis has appeared and the supracolumellar chondrification has fused to the columella auris as the medial supracolumellar process (Crompton, 1953), which corresponds both to the dorsal process and to the medial part of the supracolumellar process mentioned in the literature. The medial supracolumellar process is present in the ostrich (*Struthio camelus*) incubated about 15 days, but the lateral supracolumellar process never develops (Brock, 1937).

In the 8.5-day duck (*Anas platyrhynchos*) embryo, the stylohyal chondrifies independently, although it is connected with the distal end of the columella auris by a thin strand of precartilaginous tissue (Beer and Barrington, 1934) representing the interhyal (Crompton, 1953). According to Sonies (1907), the interhyal soon chondrifies, becoming the infracolumellar process, but Beer and Barrington (1934) stated that it has disappeared in the 9-day embryo. There is a small knob at the ventral end of the stylohyal (Sonies, 1907). In the 9.5-day duck (*Anas platyrhynchos*) embryo (Sonies, 1907; Crompton, 1953), the pharyngohyal and epihyal portions of the columella auris are fused, and the extracolumellar process has developed from the lateral surface of the epihyal. The lateral supracolumellar process (that is, the lateral portion of the supracolumellar



arcade) has chondrified and has fused with the epihyal part of the columella auris, or, according to Sonies (1907), with the extracolumellar process. It is also fused with the medial supracolumellar process, so that the foramen of Huxley is enclosed in cartilage. In the 15-day ostrich (*Struthio camelus*), the lateral supracolumellar process is ligamentous (Brock, 1937).

In the chick, the chondrification of the columella auris takes place during the seventh and eighth days. According to Smith (1904), the footplate and rod of the columella chondrify in continuity with the auditory capsule on the seventh day and separate from it on the eighth; Lutz (1942) could not corroborate this continuity. An independent ventral nodule, apparently corresponding to the knob of the stylohyal in the duck (*Anas platyrhynchos*) also chondrifies on the seventh day (Smith, 1904; Sonies, 1907). After the extracolumellar process appears, the stylohyal and the infracolumellar process chondrify together, but independently of the columella. The infracolumellar process then fuses with the columella, thus connecting the latter with the stylohyal (Sonies, 1907); this situation is found in the ostrich (*Struthio camelus*), also (Brock, 1937). The supracolumellar process (or processes, for a foramen of Huxley is present in the chick) chondrifies last. The separate knob of the stylohyal is still present in the 10-day embryo (Sonies, 1907). The stylohyal also appears as two independent chondrifications in the starling, *Sturnus vulgaris* (Crompton, 1953).

**The Third Visceral Arch—the Hyobranchial Skeleton.** The third visceral (first branchial) arch provides the main portion of the hyobranchial skeleton, which supports the tongue and its associated muscles. The hyobranchial skeleton consists of a longitudinal median portion, or copula, and the paired cornua. The copula lies in the base of the tongue anterior to the larynx and is composed of copula I anteriorly and copula II posteriorly; the two are usually joined by cartilage. Copula I articulates orally with an additional median structure known as the entoglossal bone or process or as the paraglossum; this extends forward into the tongue and may be either bony or cartilaginous. The cornua are long, thin structures attached to the copula at the junction of copula I and II and extending obliquely backward at an acute angle. Usually their anterior portions are curved, with concavity directed medially, and their free posterior ends turn dorsalward. Each cornu is composed of two bones, an anterior element known as the ceratobranchial and a posterior element termed the epibranchial (Parker, 1869). Among different species, there is considerable variation in the relative lengths of the component parts of the hyobranchial skeleton.

The anlagen of the hyobranchial skeleton were seen in the 43-mm. penguin (*Spheniscus demersus*) embryo by Crompton (1953). At this stage, copulas I and II consist of local concentrations in a dense mass of blaste-



matous tissue. The cornua are represented by a pair of elongated blastemas in the third visceral arch (cf. Fig. 353-A; Fig. 360-A). These are connected to copula II, which, as indicated by Parker (1869), is a basibranchial. Attached to the posterodorsal edge of each cornu is a faint tract of blastematous tissue designated more or less arbitrarily as a pharyngobranchial. As previously mentioned the blastematous "hypohyals" of the hyoid arch are connected at this stage with copula I. This relationship indicates that Parker (1869) was correct in designating copula I of the chick as a basihyal.

The first element of the hyobranchial skeleton to chondrify is copula I, which appears in the chick or duck (*Anas platyrhynchos*) at the same time as the quadrate, that is, during or at the end of the sixth day of incubation; in the ostrich (*Struthio camelus*), copula I is still blastematous when the quadrate chondrifies (Brock, 1937). During the chick's seventh day and the duck's eighth, copula II appears behind copula I and soon fuses with it (Sonies, 1907; Beer and Barrington, 1934); in the 47-mm. penguin, *Spheniscus demersus* (cf. Fig. 353-B; Fig. 360-B), copula I and II are both chondrified and in continuity, though still distinguishable from each other. The oral end of copula I is extended forward by a blastematous process in the 11-mm. duck, the 5- or 6-mm. sparrow, *Passer domesticus* (Kallius, 1905), and the 47-mm. penguin (Crompton, 1953). This process soon disappears either by atrophy (Crompton, 1953) or possibly (in the kestrel, *Falco tinnunculus*) by fusion with copula I (Suschkin, 1898); hence it is not the primordium of the paraglossum, but may be homologous with the lingual process of reptiles (Crompton, 1953).

The ceratobranchials are precartilaginous in the 7-day duck (*Anas platyrhynchos*) embryo and chondrifying in the embryo incubated 7.5 days (Beer and Barrington, 1934). In the chick embryo, they chondrify early in the seventh day (Sonies, 1907).

In the 56-mm. penguin (*Spheniscus demersus*) the pharyngobranchials are cartilaginous and isolated, and two centers of chondrification have appeared in each blastema of the third arch (cf. Fig. 353-C; Fig. 360-D); the anterior center represents the ceratobranchial and the posterior center represents the epibranchial. The epibranchials of the chick and duck (*Anas platyrhynchos*) begin to chondrify 24 hours or more after the ceratobranchials (Sonies, 1907; Beer and Barrington, 1934). Kallius (1905) believed that the epibranchials and ceratobranchials become distinct from each other in later stages through the segmentation of a solid cartilaginous branchial arch. According to Sonies (1907), on the contrary, these elements sometimes fuse together as development advances.

About at the time that the epibranchials chondrify, the hyobranchial skeleton migrates forward so that the copula comes to lie beneath the trabeculae (Crompton, 1953) and the posterior ends of the cornua move



up from the level of the middle of the quadrate cartilages to that of their anterior ends; simultaneously, the angle between the cornua becomes more acute. This forward migration continues until the anterior end of the copula lies at a level halfway between the anterior and posterior ends of Meckel's cartilage (Fig. 360-E). (In the sparrow, *Passer domesticus*, which has a short beak, the change in the position of the hyobranchial skeleton relative to Meckel's cartilage is less noticeable.) The cornua now begin to lengthen caudad (Kallius, 1905). By the duck's (*Anas platyrhynchos*) fourteenth day of development, they are greatly elongated and curve upward behind the skull (Beer and Barrington, 1934). Endochondral ossification of the cornua begins in their anterior portions and proceeds in both directions from the point of origin (Kallius, 1905).

As soon as the copula has reached its most anterior position, the blastematos anlagen of the paraglossum become apparent. These are seen in the 61-mm. penguin (*Spheniscus demersus*) embryo, in the form of a narrow median plate and two lateral condensations, one on each side of the plate. The median plate lies dorsal to the oral end of the copula and merges anteriorly with the remnant of the lingual process, whose contribution to the paraglossum is uncertain. Much the same stage is seen in the 71-mm. duck (*Anas platyrhynchos*), in which the paired anlagen are fused posteriorly, dorsal to the copula; and in the 5- or 6-mm. sparrow (*Passer domesticus*), in which the paired anlagen diverge posteriorly (Kallius, 1905). Chondrification of the paraglossum has begun in the 23.5-mm. sparrow (Kallius, 1905), the 8-day chick, the 9-day duck (Sonies, 1907), and the 74-mm. penguin (Crompton, 1953). In the chick and the sparrow, the paired portions of the paraglossum diverge posteriorly. Near the time of hatching, the paraglossum of the duck becomes a broad, flat, rectangular plate from which a narrow median process extends forward into the tip of the tongue. The posterior end of the duck's paraglossum articulates with the slanting anterodorsal end of the copula. The lateral portions of the newly hatched sparrow's paraglossum curve medially to articulate with the dorsal surface of the copula and are united anteriorly by a median cartilaginous plate (Kallius, 1905).

Many investigators have regarded the paraglossum as a derivative of the hyoid arch. The "lower ends of the hyoid arch" have been considered as producing either the entire paraglossum (Parker, 1869; Suschkin, 1898) or its posterolateral portions (Reichert, 1837). It has also been stated, more specifically, that the paraglossum consists of fused ceratohyals (Parker, 1866; Sonies, 1907; Beer and Barrington, 1934) or of ceratohyals fused with the basihyal (Parker, 1892a). However, Crompton's (1953) observation of a connection between the blastemas of copula I and the stylohyal seems to confirm Kallius' (1905) assertion that the paraglossum is not derived from the hyoid arch but is a new structure peculiar to birds.



**The Fourth Visceral Arch.** In the penguin (*Spheniscus demersus*), a small blastematous anlage of the fourth visceral (second branchial) arch is found on each side behind the third arch of the 43-mm. embryo (cf. Fig. 360-A), but it is no longer present in the 47-mm. embryo (Crompton, 1953). Kallius (1905) described a pair of transitory blastemas attached to the posterior end of the precartilagenous copula II of the duck (*Anas platyrhynchos*) and the sparrow (*Passer domesticus*). On the other hand, Beer and Barrington (1934) suggested that the third visceral arch is represented by a pair of cartilages present in the 14-day duck embryo just behind the copula and embracing the glottis.

### *Ossification of the Skull*

Deposition of the bony tissue which will form the skull of the adult bird begins in the "membranes" of the head of the embryo before the chondrocranium is complete. These dermal bones calcify more rapidly than do replacement bones, and most of them are complete in the chick by the sixteenth day of incubation.

The order of ossification of all the bones of the skull is given in Table 22 for the chicken (*Gallus gallus*) and the great skua (*Catharacta skua*).

**Membrane Bones.** The chief membrane bones are those of the vault of the skull such as the frontal, parietal, and squamosal bones and the bones in the facial region.

**The maxillary region.** In all species studied, the first bony deposit is found in the anlage of the quadratojugal, which appears on the seventh (Erdmann, 1940) or the tenth (Schinz and Zangerl, 1937) day of incubation in the chick, and on the tenth day for both the great skua, *Catharacta skua* (Maillard, 1948), and the pigeon, *Columba livia* (Schinz and Zangerl, 1937). In the chick, according to Erdmann (1940), a small spear of bone in the dorsolateral portion of the membrane grows both anteriorly and posteriorly, reaching the quadrate cartilage during the eighth day. A bony plate appears in the posterior three quarters of the jugal bone on the ninth day; at the same time, the anterior portion of the jugal process of the maxillary bone is formed (Fig. 361-A). The quadratojugal, jugal, and maxillary plates are in continuity by the end of the ninth day, and are beginning to fuse into a rod which will be the major part of the upper jaw. The maxillary element develops a dorsal extension, the process ascendens nasalis, which will occupy the ventrolateral angle of the nasal cavity. The palatine process of the maxillary bone appears at 10 days, projecting medially and posteriorly to form an angle of about 30° with the jugal process (Fig. 361-B); it will be extended medially into the pars palatina in the following days. In the great skua, the body of the maxillary is thickened at the junction of the nasal and jugal processes to provide support for the heavy curved beak (cf. Fig. 361-D).



TABLE 22  
Time of Initial Ossification of the Avian Embryo

Bone	Chick, <i>Gallus gallus</i>		Skua, <i>Catharacta skua</i>
	Erdmann (1940)	Schinz and Zangerl (1937)	Maillard (1948)
	(days)	(days)	(days)
Quadratojugal	7	10	10
Squamosal	7	10	13
Pterygoid	7	11	14
Supra-angular	7	10	14
Nasal	7	11	14
Ceratobranchial	7	12	16
Angular	8	11	15
Maxillary	8	12	14
Palatine	8	11	14
Quadrate	8	12	19
Basisphenoid	8	11	14
Premaxillary	8	11	14
Jugal	8	12	15
Splénial	8		15
Lacrymal	8	11	15
Dentary	8	12	15
Frontal	8	12	15
Exoccipital	9	12	16
Mentomandibular	9		
Basitemporal	10	12	16
Parietal	10	13	23
Gonial	10		
Vomer	11		15
Supraoccipital	11	13	21
Basioccipital	11	13	18
Articular	14		29
Sclerotal		16	24

Erdmann (1940) finds that the earliest indication in the chick of the premaxillary bone, which will form the beak, is a small osseous plaque on the rostradorsal surface of the membranous anlage of the prenasal process, although in the great skua, *Catharacta skua* (Maillard, 1948), the maxillary apophysis, located in the jaw, appears first (at 14 days). The nasal process extends posterodorsally as the front of the beak. At about 10 days (chick) or 18 days (skua) ossification of a palatine process is proceeding posteriorly from the ventromedial angle of the premaxillary (Fig. 361-B). By the middle of the eleventh day (cf. Fig. 361-C), the chick has a well-formed beak; that of the skua is complete by the eighteenth day of incubation.



The dermal contribution to the nasal capsule is completed with the ossification of the nasal bone, which fuses in the dorsal side of the beak with nasal processes of the frontal and premaxillary and ventrally with the apophysis of the maxillary, thus building part of the roof and the posterior border of the capsule. Erdmann (1940) observed a nasal rudiment in the

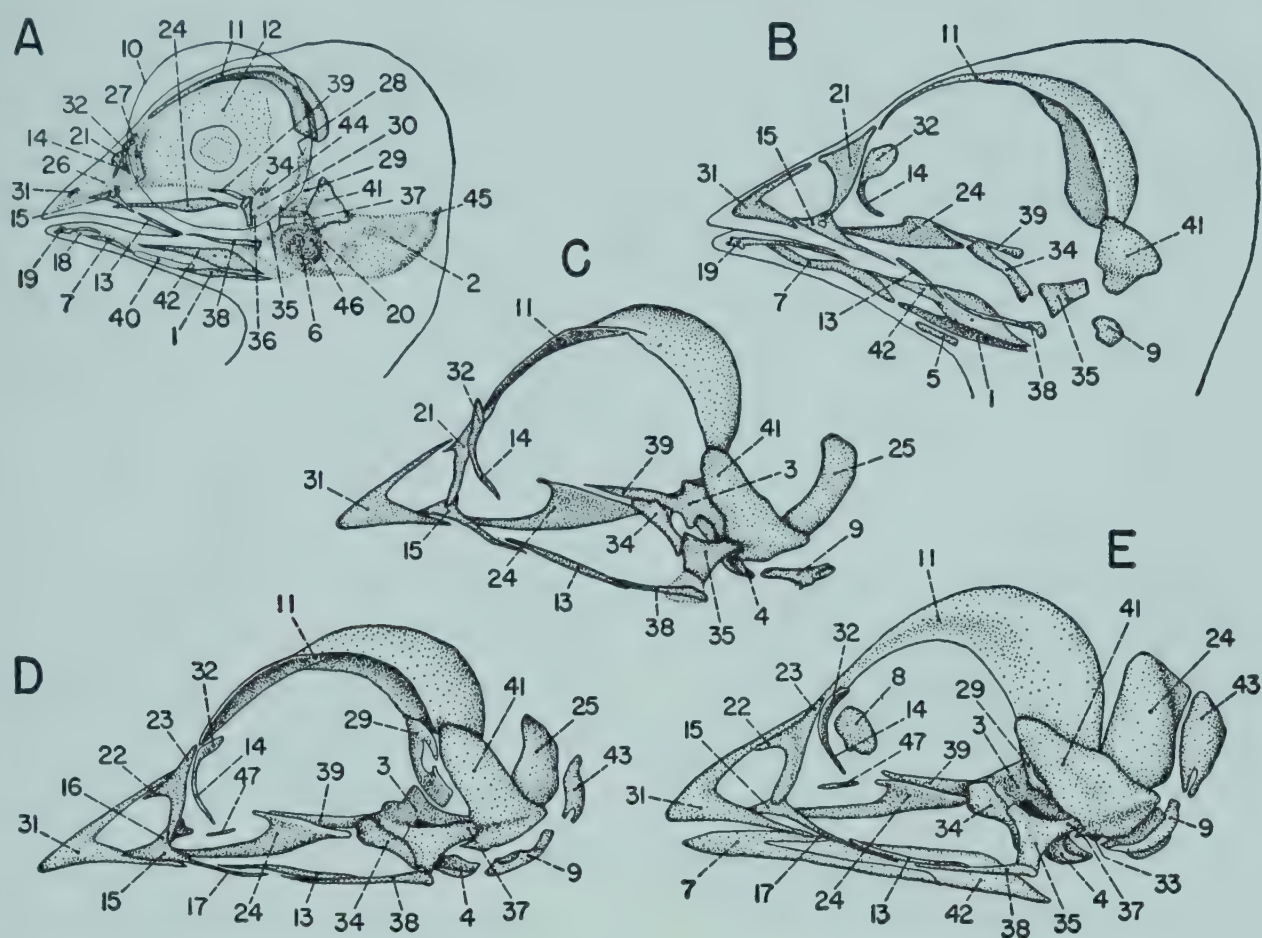


Fig. 361. Successive stages in the ossification of the skull during the second week of development of the chick. (Redrawn with modifications after Erdmann, 1940.)

A through E, lateral views of the chick skull at 9.5, 10.33, 11.5, 13.5, and 14.5 days of incubation.

1, angular; 2, auditory capsule, pars canaliculi; 3, basisphenoid; 4, basitemporal; 5, ceratobranchial; 6, columella auris; 7, dentary; 8, ethmoid; 9, exoccipital; 10, edge of eyeball; 11, frontal; 12, interorbital septum; 13, jugal; 14, lacrimal; 15, maxillary; 16, maxillary, process ascendens nasalis; 17, maxillary, jugal process; 18, Meckel's cartilage; 19, mentomandibular; 20, metotic cartilage; 21, nasal; 22, nasal, premaxillary process; 23, nasal, frontal process; 24, palatine; 25, parietal; 26, paranasal cartilage; 27, planum antorbitale; 28, planum sphenolaterale; 29, pleurospenoid; 30, polar cartilage; 31, premaxillary; 32, prefrontal; 33, pro-otic; 34, pterygoid; 35, quadrate; 36, quadrate, orbital process; 37, quadrate, otic process; 38, quadratojugal; 39, rostrum of parasphenoid; 40, splenial; 41, squamosal; 42, supraangulare; 43, supraoccipital; 44, suprapolar cartilage; 45, synotic tectum; 46, tympanic cavity; 47, vomer.

chick at 7.5 days as a triangular plate in the posterior dorsal wall directly behind the paranasal cartilage (cf. Fig. 361-A); in the great skua (*Catharacta skua*), deposition of bone begins ventrally in the maxillary process (Maillard, 1948). By the chick's ninth day *in ovo*, a curved extension of the ventral corner of the triangle has reached the maxillary, growing laterad to its process ascendens nasalis, and on the tenth day the frontal



process is approaching the border of the frontal bone. A day later, the dorsorostral corner is sending a sharp spike toward the premaxillary. Growth of this element in the following days is a matter of increase in thickness and fusion with adjoining bones. The nasal bone of the skua is fused with the prefrontal and maxillary by the twentieth incubation day, and with the premaxillary at the time of hatching.

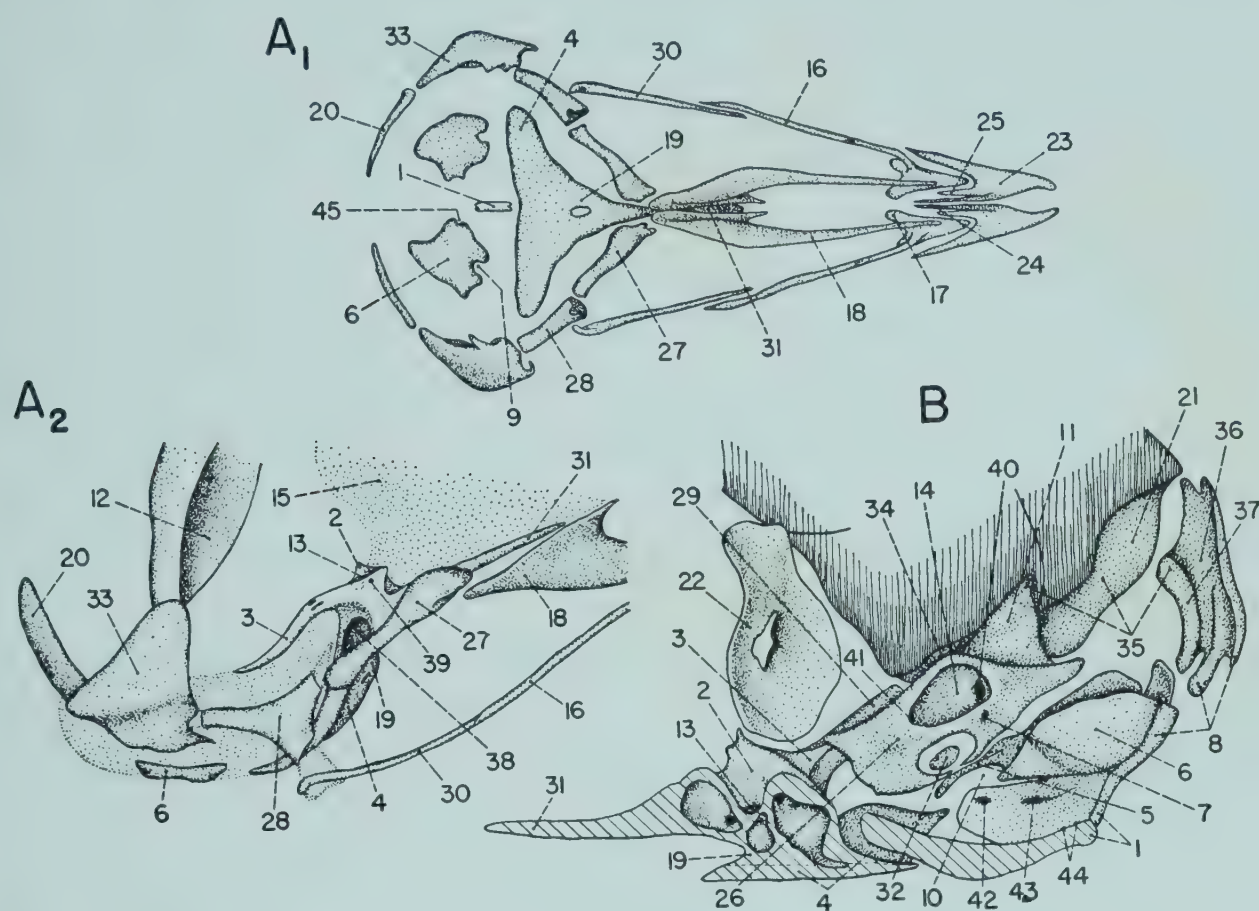
**The prefrontal region.** The prefrontal and lacrymal bones appear on the ninth incubation day, the former as a triangle following the bulge at the rostrodorsal margin of the eye, the latter as a small needle lying at the anteroventral rim of the orbit (Erdmann, 1940). About 8 hours later the two rudiments have fused (cf. Fig. 361-A). The complex grows considerably in length, forming the rostral corner of the orbit (cf. Fig. 362-B). It becomes joined to the overlapping margin of the frontal on the sixteenth incubation day and to the nasal after hatching; Maillard (1948) describes the nasofrontal junction as occurring somewhat earlier in the skua (*Catharacta skua*).

**The cranial vault.** The major part of the vault of the skull is constructed of the frontal, parietal, and squamosal bones. Erdmann (1940) finds the anlage of the squamosal in the chick incubated for 7.5 days as a loose network of bony trabeculae just posterior to the quadrate. At 9 days the frontal is represented, according to Erdmann (1940), by two well-separated ossification centers, the anterior one dorsolateral to the upper edge of the interorbital septum near its center, the posterior one in what will be the caudal third of the adult bone (designated by Erdmann, 1940, as the postfrontal). Schinz and Zangerl (1937) described a bone similar to that in Fig. 361-A appearing first at 11 days. Erdmann (1940) states that the centers are continuous by the middle of the ninth day, extending on the tenth day as far forward as the nasal bone and adjoining the squamosal posterolaterally. The latter element is observed to be roughly triangular in shape, having its ventral corner lateral to the otic process of the quadrate and the side of the ear capsule (cf. Fig. 361-B). The median frontal suture begins on the tenth incubation day, according to this author. By the middle of the eleventh, the shape of the adult squamosal has been established (cf. Fig. 361-C and D); it is subsequently joined to the otic process of the quadrate by deposition of new bone (Erdmann, 1940). The rudiment of the parietal has appeared (at 10.5 days) dorsolateral to the upper edge of the ear capsule. It has several ossification centers in the skua (*Catharacta skua*) incubated 23 days (Maillard, 1948). By the thirteenth day it has grown medially to meet the parietal of the opposite side (Fig. 362-A<sub>1</sub> and A<sub>2</sub>); the ventral and posterior two thirds of the adult bone is formed. One day later the parietal has reached dorsally to meet the frontal, and its anterior edge has extended beneath the squamosal. Fusion of the elements of the cranial vault now begins and is finished on



the sixteenth day when the frontal suture reaches its posterior limit (*Erdmann, 1940*).

*The palate.* The membranous roof of the mouth is composed medially and anteriorly of the palatine and the palatine apophyses of the maxillary and premaxillary bones; a posterior supporting element is furnished by the pterygoid. Erdmann (1940) has observed that the pterygoid ossifies from



**Fig. 362.** Later stages in ossification of the chick skull. (Redrawn with modifications after Erdmann, 1940.)

A<sub>1</sub>, ventral aspect of cranium of 11.5-day embryo; A<sub>2</sub>, detail of the floor of the brain case from the right side, the basi- and pleurosphenoids are not shown; B, right side of bony floor of the cranium from a 15.5-day embryo, showing auditory capsule, from the inside by means of a sagittal section through rostrum, sphenotemporal complex, and basioccipital. Spaces are occupied by cartilage. Anterior end is to the left.

1, basioccipital; 2, basisphenoid; 3, tympanic wing of basisphenoid; 4, basitemporal; 5, bridge between fenestra ovalis and foramen perilymphaticum; 6, exoccipital, including capsule of posterior ampulla, ossified from it; 7, foramen endolymphaticum; 8, margin of foramen magnum; 9, foramen metoticum; 10, foramen perilymphaticum; 11, fossa subarcuata; 12, postfrontal; 13, hypophyseal groove; 14, internal auditory meatus; 15, interorbital septum; 16, jugal; 17, palatine process of maxillary; 18, palatine; 19, parasphenoid; 20, parietal; 21, margin of medial portion of parietal, which encloses the lateral part of the upper semicircular canal; 22, pleurosphenoid; 23, premaxillary; 24, prenasal process of premaxillary; 25, palatine process of premaxillary; 26, pro-otic; 27, pterygoid; 28, quadrate; 29, otic process of quadrate; 30, quadratojugal; 31, rostrum of parasphenoid; 32, medial plate of stapes; 33, squamosal; 34, inner groove in squamosal for horizontal semicircular canal; 35, bony wall around superior semicircular canal; 36, supraoccipital; 37, ventral bend of superior semicircular canal; 38, lateral foramen for carotid artery; 39, foramen for ophthalmic artery; 40, foramen for superior vestibular ramus of cranial nerve VIII; 41, 42, 43, 44, and 45, foramina for nerves VII, IX, X, XI, and XII, respectively.



a lancet-shaped center in the middle of its presumptive length, growing diagonally and broadening at either end. Medially, it approaches the rostrum parasphenoidae on the eleventh day, and reaches the orbital process of the quadrate laterally. The palatine has appeared late on the eighth day, as a needle of bone just in front of the pterygoid (Erdmann, 1940). The ossifying plate reaches the nasal capsule within a day; its caudal end grows medially and dorsally so as to apply itself to the side of the rostrum parasphenoidae. At 14 days Erdmann (1940) finds the pterygoid fused posterolaterally to the quadrate, and during the following day this worker notes that union occurs between all the elements of the palatine complex.

**The ethmoid and septal regions.** The vomer of the chick is laid down on the twelfth day (Erdmann, 1940) in the ventral part of the nasal septum at the level of the division between orbital and nasal cavities. It lies free in the septum and remains rudimentary (Fig. 361-D and E). The skua (*Catharacta skua*), however, has a well-developed vomer which is fused with the premaxillary at its oral extremity and with the palatine at its aboral end (Maillard, 1948).

**Ossification of the Chondrocranium.** Replacement of the cartilaginous embryonic brain pan begins in the otic process of the quadrate. This element, which articulates with the lower jaw, is cartilaginous only at its ends by the chick's fourteenth day (Erdmann, 1940).

**The sphenotemporal region.** In the chick incubated for 9 days, Erdmann (1940) observed formation of the sphenoid complex beneath the interorbital septum, trabeculae, and infrapolar cartilages. The sphenoids have been considered to be membrane bone (Parker, 1869; Tonkoff, 1900b), but study of microscopic sections of early stages in their formation (Haardick, 1941; Kesteven, 1942) has revealed that the bony lamina which ossifies on the ventral surface of the trabecular cartilage quickly becomes connected with endochondral trabeculae of bone. This is the basisphenoid; its exact location varies with different species, and according to Erdmann (1940) even between two breeds of chickens. In the embryo of the Rhode Island Red (*Gallus gallus*), the centers are paired, lying lateral to the hypophyseal fenestra and ventral to the foramen for the ophthalmic artery. The anlage is bilateral also in the skua (*Catharacta skua*), where it was first observed at 15 days of incubation by Maillard (1948). In White Leghorns (*Gallus gallus*), however, Erdmann (1940) finds that the anlage is represented by a small median ossification in the anterior wall of the hypophyseal fenestra, just posterior to the growing rostral center of the complex; it is similarly unpaired in the emu (*Dromaeus novae-hollandiae*), occurring first as a plate on the ventral surface of the wall of the hypophyseal fossa. A second sphenoid center is located in the posterior ventral portion of the interorbital septum, extend-



ing from the plane of the posterior ends of the forming palatines to the anterior margin of the hypophyseal fossa (Erdmann, 1940; Kesteven, 1942); this will give rise to the rostral part of the parasphenoid (presphenoid). The parasphenoid and basisphenoid centers produce a continuous bony plate extending on the tenth day of incubation from the anterior wall of the hypophyseal fossa into the posterior part of the interorbital septum. At this time Erdmann (1940) has found accessory membranous centers in connective tissue on either side of and ventral to the hypophyseal foramen and between the infrapolar cartilages; these will later connect the lateral wings of the parasphenoid with the basitemporal. Caudal to the posterior segment of the parasphenoid in the polar cartilage beneath the hypophyseal cavity, there appear the paired primordia of the basitemporal bone (Erdmann, 1940). These replacement centers have been identified also by Maillard (1948) in the 16-day skua (*Catharacta skua*). On the chick's eleventh day, a new ossification has been observed by Erdmann (1940) on the dorsal surface of the cartilage joining the hypophyseal fossa and the pars cochlearis; this will be the tympanic wing of the basisphenoid. At 11.5 days in the chick (Erdmann, 1940) and 21 days in the great skua (Maillard, 1948), the basitemporal is unified with the sphenoid complex, contributing the two basal corners of a triangular plate, which curve posteriorly and laterally toward their later articulation with the squamosal (Fig. 361-C and E). At thirteen days the mediorostral end of the tympanic wing has become attached to the basisphenoid (cf. Fig. 361-D); proceeds to grow in a caudal direction, forming the dorsal wall of the recessus cochlearis and fusing at its caudal extremity with the prootic. The basitemporal contributes the ventral and rostral walls of the recessus cochlearis (cf. Fig. 362-B). Fusion of basitemporal and basioccipital does not occur until growth of the skull has ceased entirely (Erdmann, 1940).

**The optic region.** The base of the interorbital septum is ossified by the rostrum of the parasphenoid (presphenoid). Kesteven (1942) described a cartilaginous growth zone which occurs dorsal to this ossification center in the emu, *Dromaeus novae-hollandiae* (Fig. 359). The dorsal bone of the septum arises from a center at the rostral corner of the eye, the ethmoid, and is not extensive in the newly hatched chick (Erdmann, 1940), but in the late emu embryo the septum is entirely reinforced by bone as far forward as the boundary of the nasal cavity (Kesteven, 1942). In the posterior wall of the orbit is the alisphenoid (pleurosphenoid or sphenolateral plate), which Erdmann (1940) finds after the twelfth day of incubation in the chick. It becomes connected to the basisphenoid only at the time of hatching. Maillard (1948) states that this bone also appears late in the development of the skua (*Catharacta skua*), about the twenty-fourth incubation day. The orbitosphenoid bone, which will furnish the



central part of the medial wall of the orbit containing the foramen for the optic nerve, is still cartilaginous at the time of hatching (Erdmann, 1940).

A ring of fifteen bones is laid down in the connective tissue of the sclera after 9 days *in ovo* in the chick and sparrow (*Passer domesticus*) according to Leplat (1912) and Hamburger and Hamilton (1951). Lemmrich (1931) states that they form on the twelfth day in the chick, and grow to overlap each other during the following 4 days. Maillard (1948) observed these plates on the twenty-fourth day of incubation in the skua (*Catharacta skua*). The number of bones in the ring ranges from ten to eighteen among the different species, the commonest number being fifteen (Lemmrich, 1931); fifteen ossicles characterize both the chick (Lemmrich, 1931) and the skua, *Catharacta skua* (Maillard, 1948).

Another scleral ossification, the os opticus, is found in about two thirds of avian species, notably in perching birds. It is formed primarily by replacement of the cartilage surrounding the optic nerve, although there is a small dermal contribution (Tiemeier, 1950). The bone develops in the third week after the chick has hatched.

**The auditory capsule.** The otic section of the chondrocranium is entirely replaced by three bones which fuse together completely some time after hatching. The pro-otic center appears in the chick in the region of the foramen for the facial nerve on the twelfth day of incubation, and, as Fig. 362-B shows, it subsequently ossifies the internal auditory meatus and the anterior and lateral semicircular canals (Erdmann, 1940; Parker, 1892a; Stresemann, 1934). The pro-otic develops in the emu (*Dromaeus novae-hollandiae*) from a center on the lateral surface of the capsule external to the horizontal semicircular canal (Kesteven, 1942). The epiotic, in the emu, begins on the outer surface of the capsule near the anterior semicircular canal; in the chick Erdmann (1940) has described a concave plate rostroventral to the facial nerve at 13 days, which forms the roof of the pars cochlearis. This author does not, however, suggest the homology of the center. The final element, the opisthotic bones, has a small center lateral to the exoccipital on the ventral surface of the capsule in the emu (Kesteven, 1942); it surrounds most of the posterior semicircular canal, and soon fuses with the exoccipital. In the chick the bony wall of the posterior canal and the connection between the fenestra ovalis and foramen perilymphaticum are formed directly from the occipital (Erdmann, 1940). The medial half of the columella has ossified in the hatched chick.

**The occipital region.** The back of the skull is closed by the occipital bones, which form a ring about the foramen magnum, encircling the spinal cord. The basioccipital (cf. Fig. 361-E) develops from a single center during the eleventh to thirteenth days of the embryonic life of the chick (Erdmann, 1940; Schinz and Zangerl, 1937); its ontogeny in the emu



(*Dromaeus novae-hollandiae*) and the skua (*Catharacta skua*) is very similar (Kesteven, 1942; Maillard, 1948). The exoccipital, dorsolateral to the basal element on either side, is present in the 10-day chick (cf. Fig. 361-B and C); during the fourteenth day (Erdmann, 1940) it ossifies the posterior part of the ear capsule (vide supra). Two supraoccipital centers, arising on the twelfth day in the chick, have united in a medial suture 2 days later, replacing the synotic tectum and forming the wall of the ventral bend of the anterior semicircular canal (Erdmann, 1940). At hatching the occipital regions of both the chick and the skua are incomplete. The rostral third of the condyle is ossified (Erdmann, 1940).

**The Lower Jaw.** The lower jaw of the chicken is composed of the dermal supra-angular, angular, splenial, and dentary; also the endochondral mentomandibular gonial (Erdmann, 1940) and articular. Erdmann (1940) notes that the first two elements appear on the eighth day in the chick, and progress rapidly in a rostral direction. On the ninth day, the primordia of the splenial appear, medial to Meckel's cartilage, the dentary, on the lateral aspect of the jaw, and the mentomandibular, replacing the cartilage near its rostral symphysis; a day later, the mentomandibulars have fused at the symphysis and Meckel's cartilage is surrounded by bone (cf. Fig. 361-B). Early in its ontogeny in some species, the dentary has rudimentary foramina for nerves and blood vessels of teeth (Schenk, 1897). The major elements of the lower jaw become continuous about the fourteenth day and undergo fusion on the sixteenth (Erdmann, 1940). The gonial, located on the medial side of Meckel's cartilage, ossifies on the tenth day of incubation; the articular, at the rostral end of the process, appears on the fourteenth day in Erdmann's preparations (1940). These two bones are beginning to fuse at hatching, but are connected by cartilage with the rest of the jaw. The mandible of the great skua (*Catharacta skua*) forms in very similar fashion, although it does not possess a gonial element (Maillard, 1948).

The ceratobranchials are the only portion of the hyoid apparatus which is ossified before hatching. In the chick (Erdmann, 1940) and the skua, *Catharacta skua* (Maillard, 1948), deposition of bone occurs exactly as it does in the long bones, beginning from a diaphysial center and leaving the epiphyseal ends cartilaginous at hatching.

### The Appendicular Skeleton

The bones of the fore and hind appendages together with their articulating structures, the pectoral and pelvic girdles, and the sternum, make up the appendicular skeleton. Except in ratite birds such as the emu (*Dromaeus novae-hollandiae*) and the ostrich (*Struthio camelus*), this part of the skeleton is highly modified for flight. Three of the five embryonic digits remain to support the distal part of the wing. The sternum bears



a keel for attachment of the heavy flight muscles; along with the humerus, cervical, and thoracic vertebrae, it has cavities for air sacs, which reduce the specific gravity of these bones (see Chapter 7). The hindlimbs are adapted to an upright stance, and the pelvic girdle is fused with the lumbosacral vertebrae at the ilium, forming the roof of the pelvic cavity. In all flying birds the first digit of the hindlimb becomes appposable to the others to provide a strong perching grip.

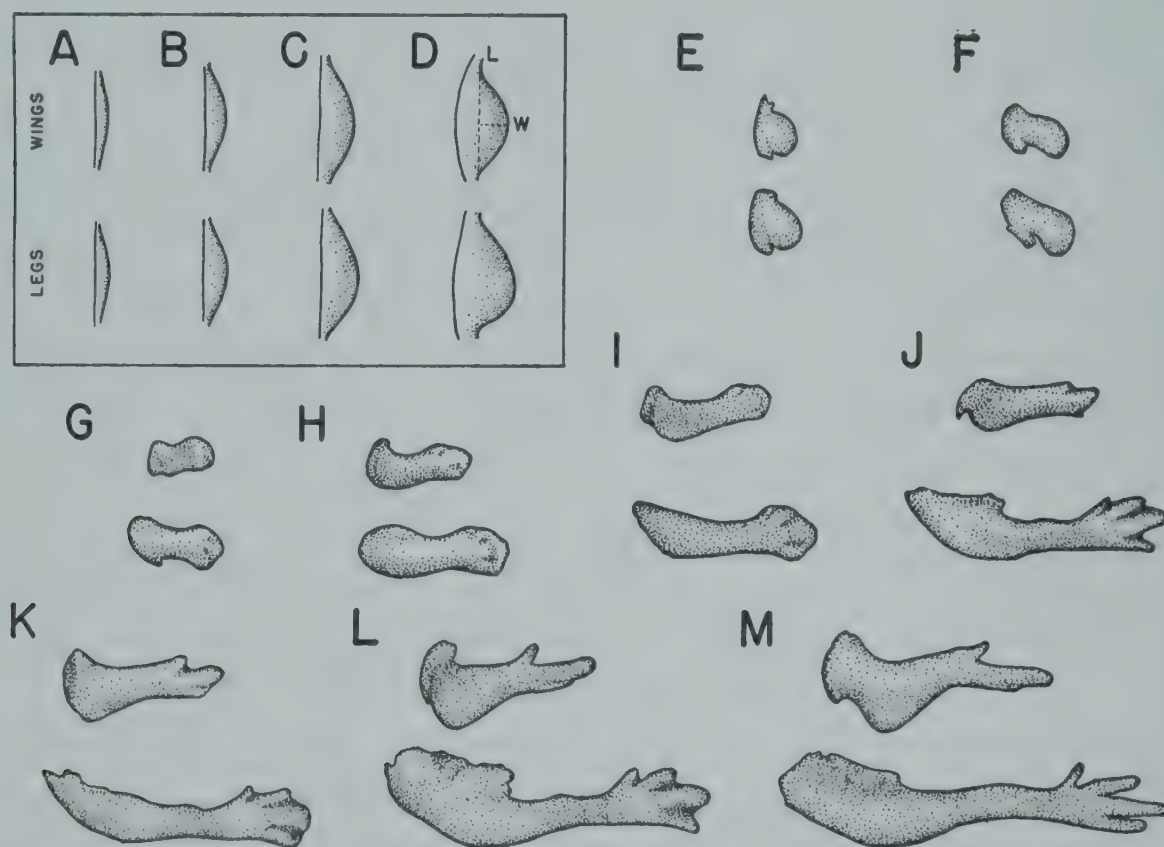


Fig. 363. Comparison of growth rates of wing and leg buds in the chick embryo, from 52 hours to 9 days. (Redrawn with modifications after Hamburger and Hamilton, 1951.)

A, wing and leg buds from 52- to 64-hour embryo ( $\times 10$ ); B, same, from 65- to 69-hour embryo ( $\times 10$ ); C, same, from 68- to 70-hour embryo ( $\times 10$ ); D, same, from 70- to 72-hour embryo ( $\times 10$ ); E, same, from 3.5- to 4-day embryo ( $\times 5$ ); F, same, from 4.5-day embryo ( $\times 5$ ); G, same, from 5-day embryo ( $\times 5$ ); H, same, from 6-day embryo ( $\times 5$ ); I, same, from 7-day embryo ( $\times 5$ ); J, same, from 7.5-day embryo ( $\times 5$ ); K, same, from 7.5- to 8-day embryo ( $\times 5$ ); L, same, from 8-day embryo ( $\times 5$ ); M, same, from 8- to 9-day embryo ( $\times 5$ ).

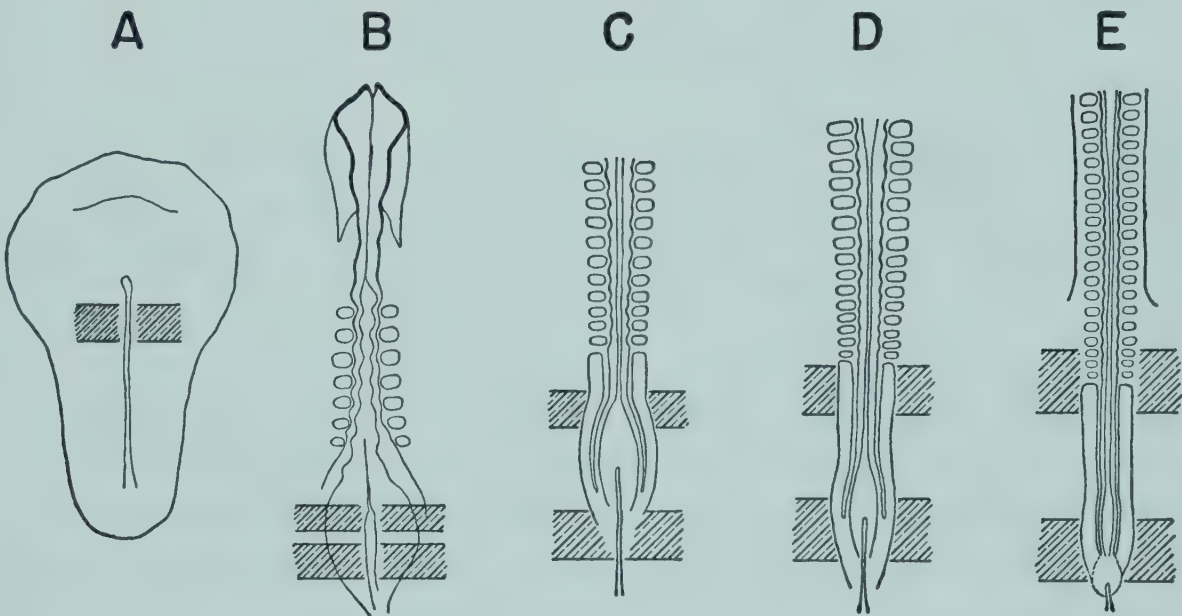
The wing bud is at the top, and the leg bud at the bottom, in each pair.

**Primordia of the Wing and Leg.** In the chick, the anlagen of the wings appear after about 50 hours of incubation (Hamburger, 1938), slightly earlier than the leg buds but almost identical to them in form (Fig. 363). The initial condensation of mesenchyme will form the girdle elements and the proximal part of the limb; the distal elements develop later from the distal blastema (Saunders, 1948; Amprino and Camosso, 1956). Skeletal elements of the leg appear earlier than those of the wing (Schmalhausen and Stepanova, 1926), and develop more rapidly; by the end of incubation, the legs are much longer than the wings as shown by



Age of Embryo (days)	Growth of Limbs	
	Forelimb (mm.)	Hindlimb (mm.)
3	4	4
5	7	7
7	12	14
9	16	22
11	20	26
13	25	35
15	32	45
17	35	50

the data of Chmutova (1949) in the accompanying table for the crow (*Corvus cornix*) and in Fig. 363 for the chick. The presumptive limb areas have been outlined by Wolff (1936a) by means of X-ray defect experiments (Fig. 364-C, D, and E). The anterior end of the wing primordium



**Fig. 364.** A diagrammatic representation of the localization of the wing and leg bud primordia at different stages in the development of the chick embryo. A and B were determined by coelomic grafts; C to E by X-ray irradiation. The wing and leg primordia are indicated by the crosshatched areas. (Redrawn with modifications A and B, after Rudnick, 1945; C to E, after Wolff, 1936a.)

A, presomite stage; B, 7-somite stage; C, 11-somite stage; D, 15-somite stage; E, 20-somite stage. All  $\times 10$ .

lies lateral to the segmental plate behind the last somite at the 15-somite stage, and lateral to the eighteenth somite at the 20-somite stage, showing a slight posterior movement (Fig. 365-E<sub>1</sub> and E<sub>2</sub>). Presumptive leg has been localized at the caudal end of the sinus rhomboidalis, coming to lie with its anterior margin laterad to the twenty-sixth somite (Murray, 1928).

**Developmental Potencies of the Presumptive Areas.** Differentiation of small limbs having only minor defects has been obtained from chorioalantoic grafts of localized areas of the blastoderm at the head-process stage (Fig. 364-A and B), after 20 hours' incubation (Rudnick, 1945). These



regions correspond rather closely with the prospective limb areas of Wolff (1936a). This is shown in Fig. 364-C, D, and E.

Dorsoventral and anteroposterior axes of both limbs have been determined by the 25-somite stage, when the primordium of the wing is just visible (Hamburger, 1938). Axiation in normal development is a property of the mesoderm rather than of the ectoderm of the bud (Zwilling, 1956a).

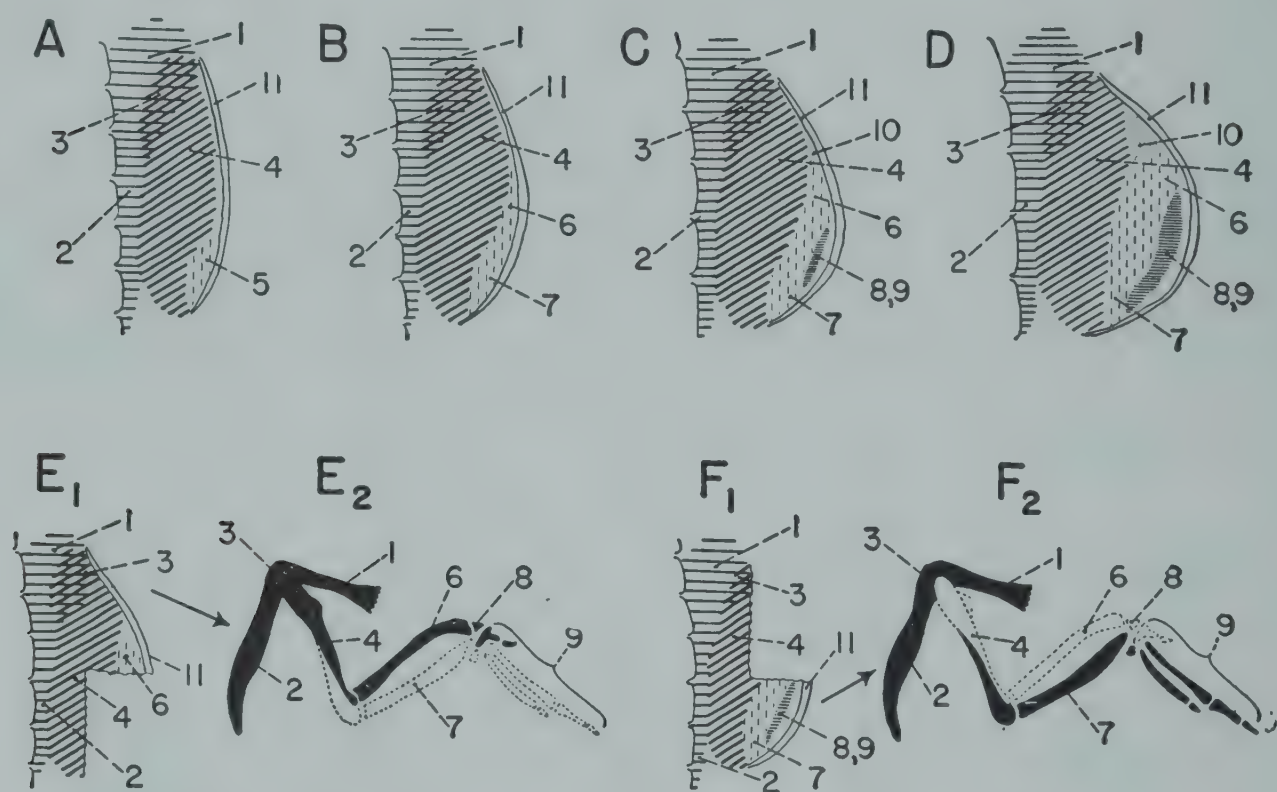


Fig. 365. Origin of the parts of chick wing; A to D show the area for the tissues of the wing parts from 18 hours to 26 hours, as traced by means of carbon marking according to defect experiments by Warren (1934). E and F show which parts form the anterior and posterior parts of the wing. (Redrawn with modifications after Saunders, 1948.)

A, 18 hours, at the definitive streak stage; B, 19 to 22 hours, at the head-process stage; C, 23 to 25 hours, at the head fold stage; D, 26 hours, 1-somite stage; E<sub>1</sub>, wing bud during the fourth incubation day with the posterior half removed; E<sub>2</sub>, diagram of wing showing the bones (stippled) that develop from the anterior portion of the wing bud; F<sub>1</sub>, wing bud during the fourth incubation day with the anterior half removed; F<sub>2</sub>, diagram of wing showing the bones (stippled) that develop from the posterior portion of the wing bud.

1, dorsal portion of coracoid; 2, scapula; 3, glenoid region; 4, upper arm; 5, forearm; 6, radial region of forearm; 7, ulnar region of forearm; 8, wrist; 9, hand; 10, web; 11, apical ectoderm.

Many experiments have been offered as evidence that the limb bud of the chick is a mosaic structure. Injury to a part of the bud prevents the appearance of corresponding regions of the limb (Lillie, 1904; Spurling, 1923; Warren, 1934; Saunders, 1948). Isolated and transplanted segments of the primordia will differentiate into the same part of the limb which they would produce normally (Murray, 1926, 1928). Saunders (1948) showed that removal of the apical ridge of the limb blastema prevents



formation of the distal parts of wing or leg. However, hindlimbs complete with all major skeletal elements can be formed from a small anterior segment of the primordium at the 27- to 29-somite stage (*Rawles, 1947*). *Hansborough (1954)* has shown conclusively that up to 50 per cent of the wing bud may be removed at 4 days without altering the morphology of the resulting appendage, providing that the apical growing region is not injured. *Zwilling (1956b)* has shown that the 3-day limb divided into two segments, an apical blastemal segment and a proximal mesenchymal one, can give rise to two reasonably complete limbs if the proximal segment be covered with limb bud ectoderm. *Rudnick (1946)* observed that many experimental limb defects can be attributed to blocked growth. According to *Zwilling (1956a, 1956b, 1956c; Zwilling and Hansborough, 1956)* morphogenesis of the appendages in the chick depends on a rather complex interaction between the two tissue components of a labile primordium, the ectoderm providing a growth stimulus, the mesoderm furnishing wing or leg specificity, axial relationship, and a factor necessary for maintaining the ectodermal ridge of the blastema.

### *The Pectoral Girdle*

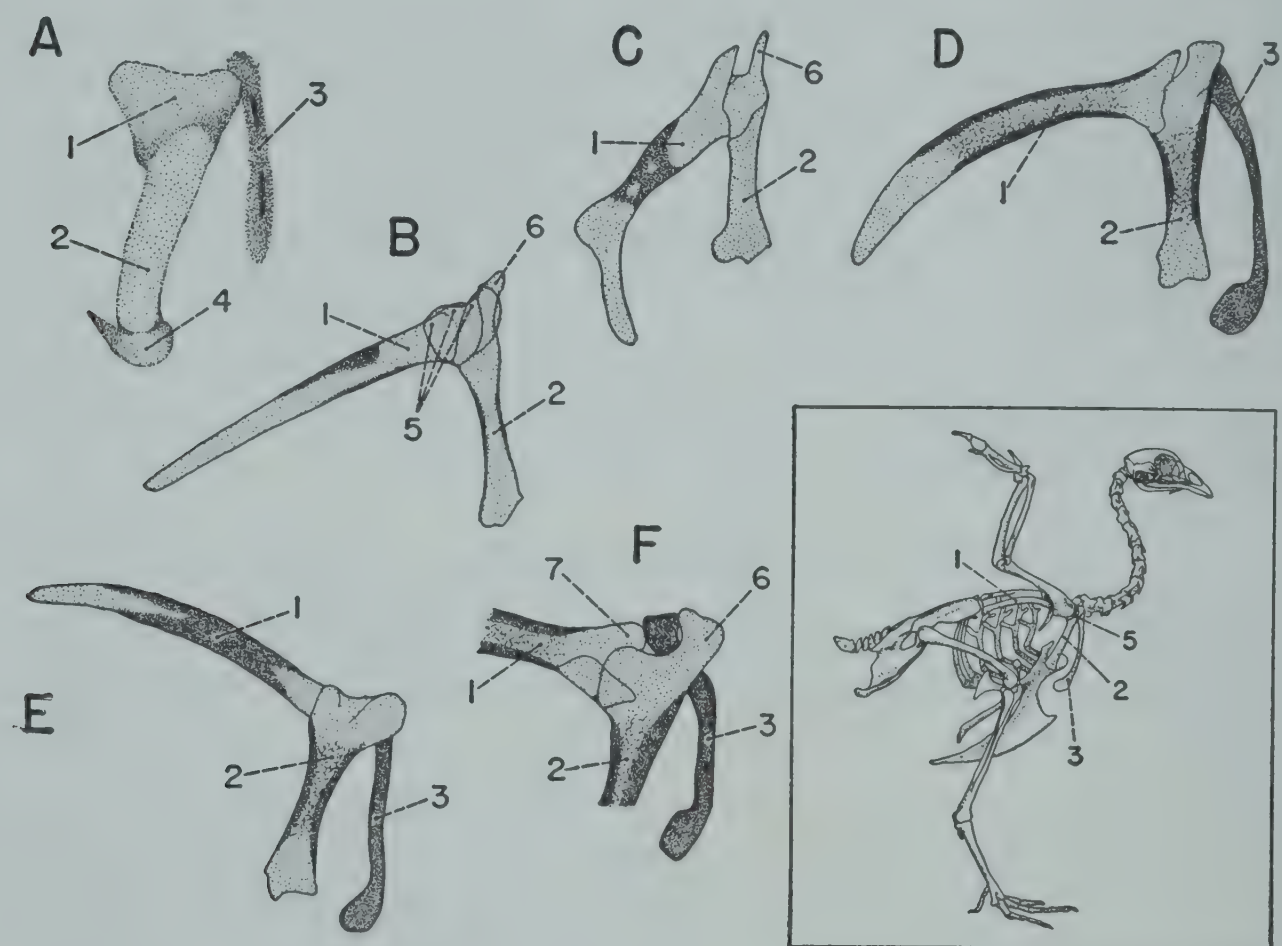
The components of the pectoral girdle are the long, narrow scapulae, running parallel to the vertebral column; the heavy coracoids articulating dorsally with the scapula and ventrally with the sternum, and the clavicles, frequently fused medially to form a furcula. The scapula and coracoid, together with the humerus, arise from a mesenchymal condensation at the base of the wing. The limits of the three elements first become distinct at the stage of cartilage formation (*Knopfli, 1919; Hommes, 1924*).

Chondrogenesis begins in the shoulder girdle during the seventh day of incubation for the chick, and on the eighth day for the duck, *Anas platyrhynchos* (*Knopfli, 1919; Hommes, 1924*). During the chick's eighth day the coracoid and scapula begin to separate, leaving a bar of precartilaginous tissue between them (*Knopfli, 1919*). The scapula elongates markedly; a depression between it and the humerus is the first indication of the glenoid cavity, its articulation with the humerus. The primordium of the clavicle is found as a strand of mesenchyme containing a very early ossification center (*Hillel, 1904; Knopfli, 1919*) as shown in Fig. 366-A. In the 10-day ostrich (*Struthio camelus*) embryo, a stage comparable to the 8-day chick (*Broom, 1906*), the coracoid and scapula are clearly outlined, but not fully chondrified.

A day later, the scapula and coracoid of the chick have the form in which they will appear in the adult, and the former element is connected to the third sternal rib. The scapula becomes bent at its distal end. An interclavicular process has appeared at the scapular end of each rapidly ossifying clavicle. During the tenth day of incubation (*Knopfli, 1919*), the scapula



and coracoid are well chondrified and perichondral ossification has begun in the scapula. It becomes completely separated from the coracoid, and migrates dorsally, its caudal end being directed posteroventrally as a result. A cleft separates the scapula from the humerus in the region which will become the articular cavity. The distal ends of the clavicles are separated at the midline of the body cavity only by connective tissue; the interclavicular process is beginning to ossify. On the thirteenth day, peri-



**Fig. 366.** Successive stages in the development of the shoulder girdle in the chick embryo. (Redrawn with modifications after Knopfli, 1919.)

A, sagittal section through the right shoulder girdle and sternum during the eighth incubation day ( $\times 10$ ); B, lateral view of the right shoulder girdle, with the individual bones shown in sagittal section, during the fourteenth incubation day ( $\times 3$ ); C, the same, during the fifteenth incubation day ( $\times 3$ ); D, the same, during the sixteenth incubation day ( $\times 3$ ); E, the same, during the eighteenth incubation day ( $\times 3$ ); F, the same, during the nineteenth incubation day ( $\times 3$ ).

*Insert:* right side of adult chicken skeleton. (Redrawn after Chamberlain, 1943.)

1, scapula; 2, coracoid; 3, clavicle; 4, part of sternal anlage; 5, glenoid fossa; 6, acromion; 7, acromion.

chondral ossification is present also in the coracoid. Three days later, the scapula and coracoid are completely separated, and the acromion and the distal fifth of the scapula are still cartilaginous. When the chick hatches these parts have ossified. The ends of the coracoid are still chondral.

Replacement of the cartilaginous shoulder girdle with bone follows a very similar sequence in the great skua, *Catharacta skua* (Maillard, 1948). The clavicle is among the first bones in the body to ossify, being complete



by 14 to 16 days, and remaining bilateral. The scapula and coracoid ossify perichondrally as in the chick.

Except for the ostriches (*Struthionidae*), some chondral elements contribute to the adult clavicle of most birds (*Parker, 1868*). The principal one of these is the precoracoid; it has been described in the chick by Goette (1877) and in the pheasant (*Phasianus colchicus*) by Parker (1868), but is evidently transitory in gallinaceous birds. Hillel (1904) has stated that the clavicle of the penguin (*Spheniscus demersus*) is a compound of the acrocoracoid, subcoracoid, and supracoracoid. Frankenburger (1942) described a procoracoid cartilage which occurs in embryos of the cormorant (*Phalacrocorax carbo*) lateral to the membranous clavicle at its dorsal end; its line of fusion with the clavicle can be detected in the adult. This bird also has a transitory interclavicle between the cranial ends of the two sternal bars, which is incorporated into the keel of the sternum.

### The Wing

**The brachium and the antebrachium.** The humerus articulates with the pectoral girdle at the glenoid fossa and furnishes skeletal support to the upper part of the forelimb. Structural elements of the distal segment of the wing are the anterior and medial radius and the lateral (posterior) ulna, which articulate proximally with the humerus and distally with the carpal bones of the wrist. The metacarpals and phalanges make up the hand and digits; in carinate birds, three of the primitive complement of five digits are found in the adult wing.

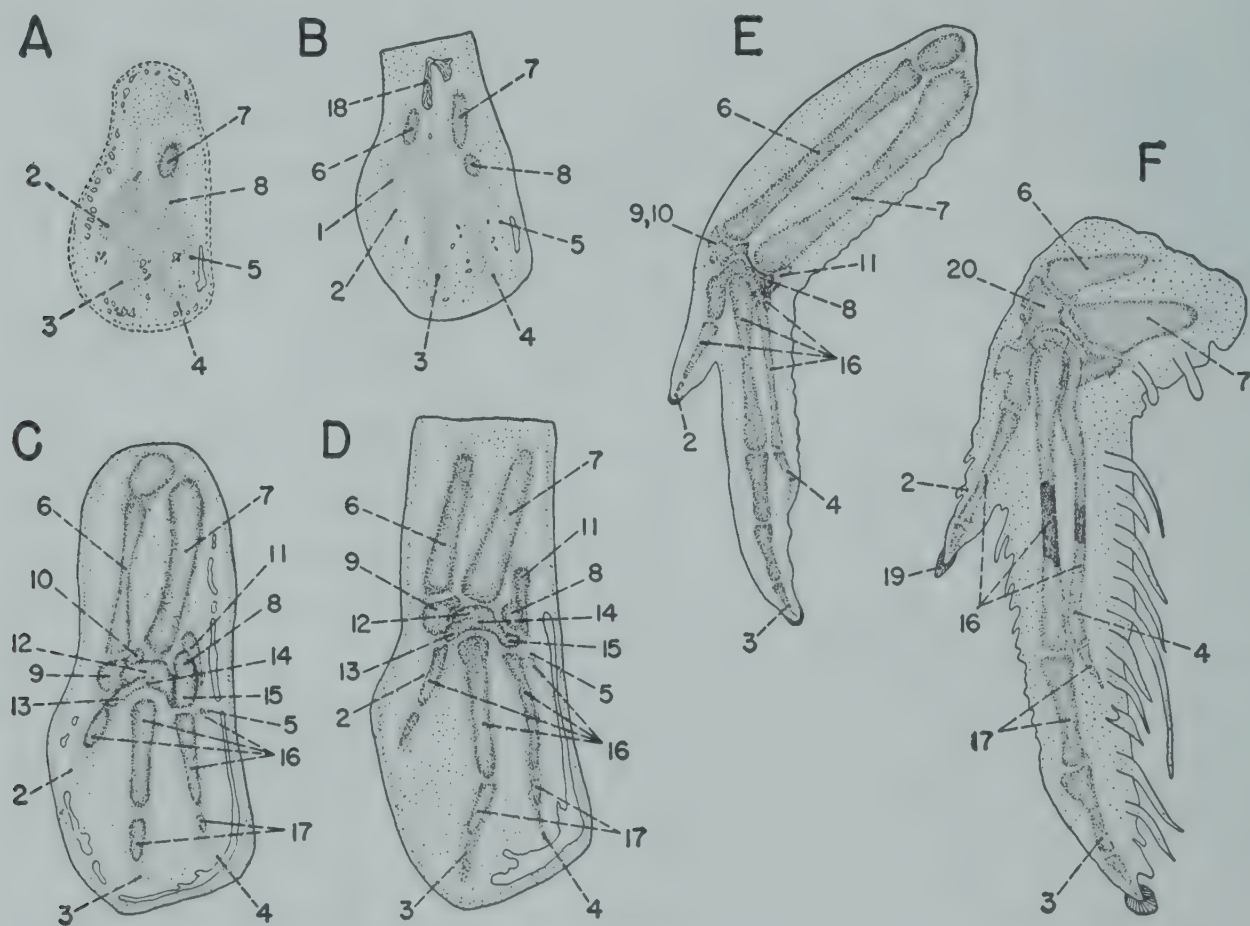
Age of Embryo (days)	Length of Humerus (mm.)	Ratio of Lengths (humerus : radius : manus)
5	0.86	1 : 0.69 : 1.29
8	1.41	1 : 0.86 : 1.71
10	1.98	1 : 1.12 : 2.31
12	2.17	1 : 1.12 : 2.32
14	2.80	1 : 1.12 : 2.30
16	3.18	1 : 1.12 : 2.36
18	3.32	1 : 1.16 : 2.36
At hatching	3.60	1 : 1.27 : 2.43
Adult	16.50	1 : 1.44 : 3.50

Chondrogenesis takes place concurrently in the humerus and the shoulder girdle at about 6 days in the chick (*Knopfli, 1919*). The humerus is separated from the coracoid and scapula by the glenoid cavity on the tenth day of incubation (*Knopfli, 1919*). An articular between humerus and radius is also apparent at this time, although a cartilaginous interzone still joins the humerus and ulna. Perichondral ossification of the humerus begins at 7 days (*Strong, 1902*), proceeding in the manner of the other long bones. In the alpine swift (*Apus melba*) the bone of the brachium increases in length, as the accompanying table shows, at a slower rate



than do the radius and manus (*Zehnter, 1890*). For many species, there is a period just before hatching in which the manus increases in length much faster than the brachium and antebrachium (*Marples, 1930*).

The radius and ulna are in a prechondral stage after 4 days of incubation in the chick, with cartilage beginning to form in the diaphyses (*Montagna, 1945*); this stage occurs at about 6 days in the duck, *Anas platyrhynchos* (*Sieglbauer, 1911*; Fig. 367-A and B). The radius is a straight rod, curved



**Fig. 367.** Stages in cartilage development and ossification of the forearm and hand of the duck embryo, *Anas platyrhynchos*. (Redrawn with modifications after *Sieglbauer, 1911*.)

A, frontal section through wing bud of 6-day embryo ( $\times 10$ ); B, composite drawing from frontal sections of 7-day embryo ( $\times 10$ ); C–F, drawn from tracing reconstructions of 8-, 9-, 12-, and 15-day embryos (C, D,  $\times 10$ ; E, F,  $\times 5$ ).

1–5, first through fifth digits; 6, radius; 7, ulna; 8, ulnare; 9, “radiale” complex; 10, intermedium; 11, pisiform; 12, central carpal region; 13, 14, distal carpals 2 and 3; 15, distal carpals 4 plus 5; 16, metacarpal element of the respective digits; 17, phalanges; 18, radial nerve; 19, cornified epidermis; 20, fused free carpals.

slightly toward the ulna at its distal end. The shaft of the ulna displays a slight outward curvature (*Montagna, 1945*). The early appearance of these curvatures indicates their independence from the influence of such extrinsic factors as muscular tension; this is proved to be the case also for the femur, which can differentiate typically in fragments of limb bud grafted to the chorioallantoic membrane (*Murray, 1926*).

Ossification in the radius and ulna of the duck (*Anas platyrhynchos*) embryo begins at about 7 days.



*The wrist.* Homologies of the carpal and metacarpal elements of *Aves* have been the subject of many investigations.

In the chick embryo of 5 days' incubation time, the wrist is composed of mesenchymal condensations which, with the exception of centrale I, have not yet separated into anlagen of the carpals. During the following 5 days, thirteen prochondral elements have been identified by Montagna (1945); they are indicated in Fig. 368-A, B, C, and D. Most of the wrist is built

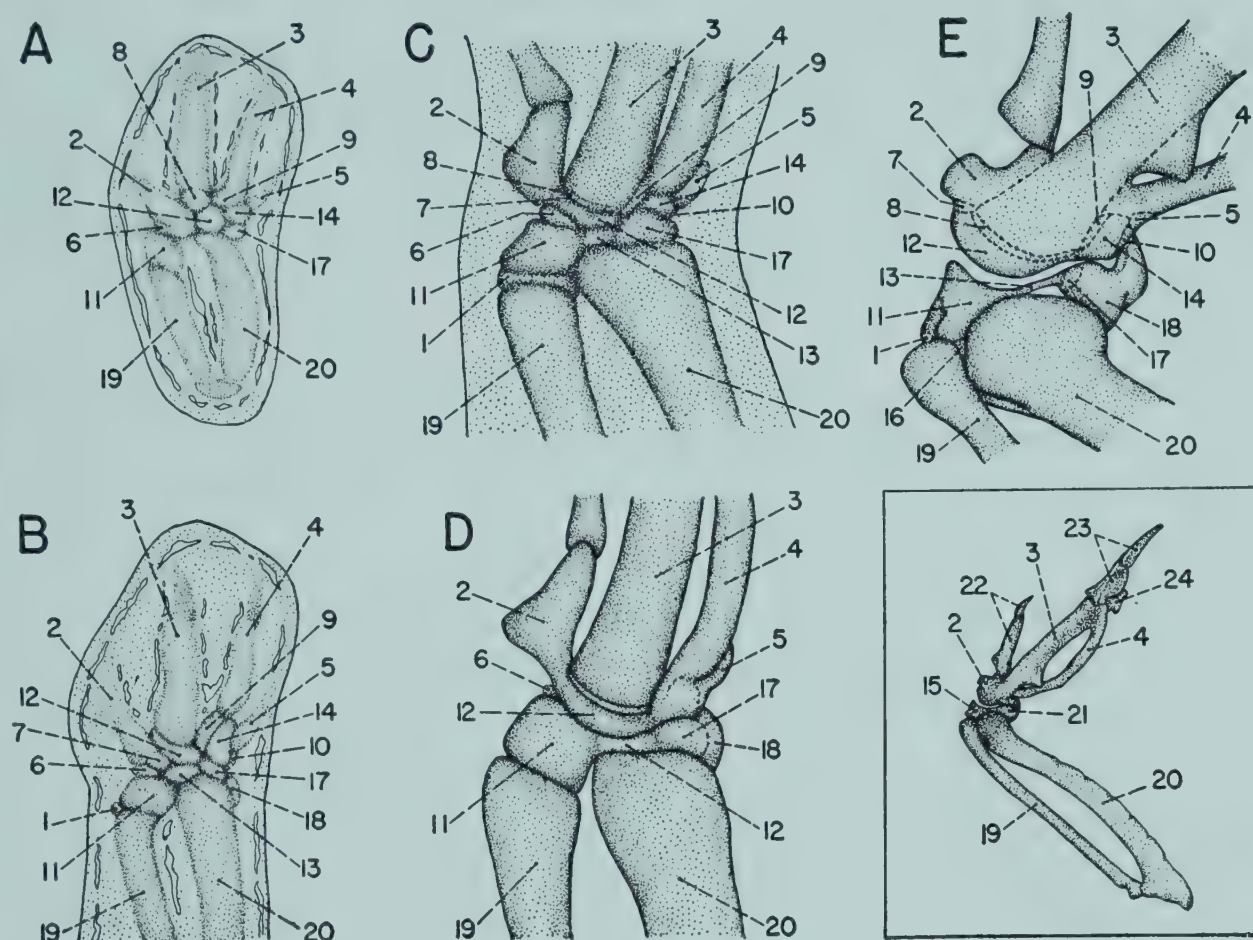


Fig. 368. Formation of embryonic cartilage elements in the wrist of the chick and their reduction and fusion during ontogeny. (Redrawn with modifications after Montagna, 1945.)

A, B, C, dorsal views of reconstructions of right forelimb of 5-, 6-, and 8-day chick embryos; D, diagrammatic representation of chick wrist at 10 to 12 days; E, diagram of adult wrist, with areas contributed by embryonic carpals indicated by dotted lines.

*Insert:* dorsal view of right wing skeleton of adult.

1, radiale; 2-5, metacarpals; 6-10, distal carpals 1-5; 11-14, central carpals; 15, radiale complex of adult; 16, intermedium; 17, ulnare; 18, pisiform; 19, radius; 20, ulna; 21, ulnare complex; 22, phalanges of digit 2; 23, phalanges of digit 3; 24, phalanges of digit 4.

from the four central carpals and the pisiform; the radiale, intermedium, ulnare, and the five distal carpals are rudimentary structures which never chondrify. Centrale I, and primordia of the radiale and intermedium fuse on the tenth day to form the free radial carpal of the adult wing (cf. Fig. 368-E and Insert). The free carpal which articulates proximally with the ulna consists of the pisiform and the rudiment of the ulnare. Joining these two free carpals is a meniscus which represents centrale III. Centrale II, together with distal carpals II and III, unites with the second metacarpal



on the twelfth day of the chick's development; similarly, centrale IV and distal carpals IV and V attach to the base of metacarpal IV on the eleventh day. Thus centralia II and IV form the proximal articular surface of the carpometacarpus. The extensive fusion of the wrist elements, particularly of those which chondrify late, has presumably been brought about by compression due to the rapid growth of the radius and ulna proximal to them, and the metacarpals on the distal side (*Montagna, 1945*).

**The metacarpals.** Primordia of the digital rays have been observed by *Montagna (1945)* as four elongate condensations of mesenchyme in the distal part of the chick's wing bud, on the fourth day of incubation. These are metacarpals II through V, the last of which is vestigial. *Montagna (1945)* identifies the "missing first digit" of *Norsa (1894)* and *Sieglbauer (1911)* as distal carpal I, and states therefore that metacarpal I is completely missing.

The second digital ray has a radial protrusion at 5 days which will become the prominent apophysis for attachment of the radial extensor of the metacarpus (*Montagna, 1945*). At 7 days, the metacarpals are chondral (*Warren, 1934*). The second digital ray grows more slowly than the others; the ratio of the lengths of II and III is approximately 1 : 2 on the seventh day, and is reduced to 1 : 5 on the ninth day (*Montagna, 1945*). Metacarpal IV begins to curve toward metacarpal III at its proximal end on the sixth day; and at about 11 days the distal end also curves radially (*Montagna, 1945*). Both ends later fuse with the third ray. The fifth metacarpal is chondrified at 7 to 8 days, but later fuses with the fourth (*Montagna, 1945*).

The emu, *Dromaeus novae-hollandiae* (*Lutz, 1942*), and the kiwi, *Apteryx oweni* (*Parker, 1892a*), have only digit III; metacarpal II disappears completely, and IV is reduced to an eminence at the base of III. The penguins, *Pygoscelis antarctica* (*Anthony and Gain, 1915*), and *Eudyptes chrysolophus* (*Hillel, 1904*), exhibit an intermediate condition in which the medial digit, presumably II, is represented only by a metacarpal. This begins to ossify, but later fuses with III, leaving two digits.

The metacarpals in the chick ossify perichondrally in order of size, the third on the seventh day of incubation, the fourth at 8 days, the second 2 weeks after hatching, and the rudiment of the fifth 1 to 2 months after hatching (*Schinz and Zangerl, 1937*).

**The phalanges.** These are chondral at 8 days in the duck (*Anas platyrhynchos*); they ossify at 12 days in the chick, according to *Schinz and Zangerl (1937)*. As the accompanying table shows, bird embryos generally have two phalanges to the second digit, two or three in the third, and one in the fourth; ratites have only those associated with the third digit. In the kiwi, *Apteryx australis* (*Parker, 1892a*), the middle phalanx on the third digit chondrifies, but does not persist in the adult in some



cases; the kiwi (*Apteryx oweni*) has an embryonic element in digit IV which later disappears. In other cases studied, phalangeal elements of reduced digits do not appear in the embryo, although the corresponding metacarpals may sometimes be found.

Species	Number of Phalanges on Digit (number in parentheses includes transient elements)					Reference
	I	II	III	IV	V	
Tern ( <i>Sterna</i> sp.)		2	3	1	1	Leighton (1894)
Chicken ( <i>Gallus gallus</i> )		2	2	1		Montagna (1945)
Kiwi ( <i>Apteryx australis</i> )			2(3)			Parker (1892a)
Kiwi ( <i>Apteryx bulleri</i> )			2-3			Parker (1892a)
Kiwi ( <i>Apteryx oweni</i> )			2-3	(1)		Parker (1892a)
Skua ( <i>Catharacta skua</i> )		1	2	2		Maillard (1948)
Tufted penguin ( <i>Eudyptes chrysolophus</i> )			2	1		Hillel (1904)
Adélie penguin ( <i>Pygoscelis adeliae</i> )			2	1		Anthony and Gain (1915)
Penguin ( <i>Pygoscelis papua</i> )			2	1		Anthony and Gain (1915)
Penguin ( <i>Pygoscelis antarctica</i> )			2	1		Anthony and Gain (1915)
Penguin ( <i>Pygoscelis papua</i> )		2	3	4	5	Sieglbauer (1911)

The Sternum

For many years the sternum was thought to originate by fusion of the ribs in the midline (Rathke, 1838; Parker, 1868; Goette, 1877; Hoffmann, 1879; Lindsay, 1885; Knopfli, 1919; Lillie, 1919). An independent origin was first suggested by Bruch (1852), confirmed in descriptive studies by Patterson (1907) and Gladstone and Wakeley (1932), and finally proved experimentally by Fell (1939). Explants of regions around and including the wing bud of the zebra parakeet (*Melopsittacus undulatus*) frequently produced a recognizable sternum in the absence of ribs and coracoid (Fell, 1939).

The bilateral mesenchymal anlagen of the sternum are located, in the parakeet (*Melopsittacus undulatus*) incubated from 6 to 7 days (Fig. 369-A and B), immediately ventral and posterior to the coracoids and continuous with them, extending posteriorly as far as the rudiment of the second sternal rib (Fell, 1939). In the 7-day chick, Hommes (1924) had found that this mesenchymal condensation is not continuous with the coracoid, which has already begun to chondrify. Precursors of the anterior lateral process and the lateral trabecula are present in the cellular rudiment in this species.

During the first day of its development in the parakeet, *Melopsittacus undulatus* (Fell, 1939), the sternal anlagen broaden in the vertical dimension, forming plates which reach posteriorly as far as the third sternal



ribs. At their anterior margins, the plates are still continuous with the coracoids, but an articular region is developing in the form of a zone of flattened cells (*Fell, 1939*). The two parts of the sternum are inclined so that their dorsal margins are closer together than the ventral margins. In 7- to 8-day embryos (Fig. 369-B, C, C<sub>1</sub>, and C<sub>2</sub>), the plates have rotated

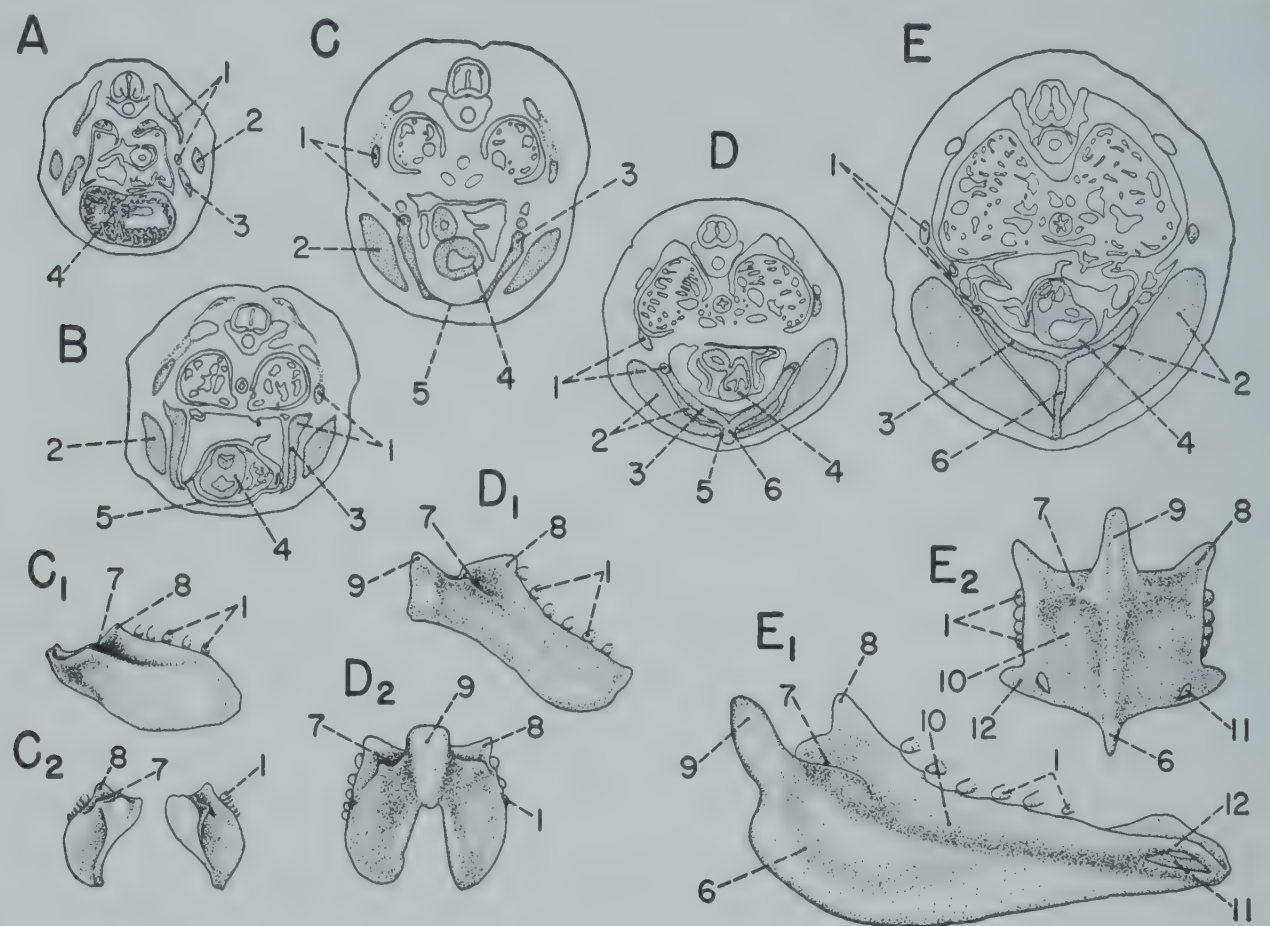


Fig. 369. The appearance of the sternal plates as they migrate from lateral to ventral positions in the zebra parakeet, *Melopsittacus undulatus*, embryo. (Redrawn with modifications after *Fell, 1939*.)

A, transverse section of a 6.5-day embryo at the level of the sternal articulation of the first sternal rib ( $\times 6$ ); B, same view of a 7-day embryo ( $\times 6$ ); C, same view of an 8-day embryo ( $\times 6$ ); C<sub>1</sub>, lateral view of sternal plate of 8-day embryo, drawn from a wax reconstruction; C<sub>2</sub>, anterior view of sternal plates, as in C<sub>1</sub>; D, transverse section of a 9-day embryo at the level of the sternal articulation of the first sternal rib ( $\times 6$ ); D<sub>1</sub>, lateral view of sternal plate of 9-day embryo, drawn from a wax reconstruction; D<sub>2</sub>, anterior view of sternal plates, as in D<sub>1</sub>; E, transverse section of an 11-day embryo, at the level of the sternal articulation of the first sternal rib ( $\times 6$ ); E<sub>1</sub>, lateral view of sternal plate of 11-day embryo, drawn from a wax reconstruction; E<sub>2</sub>, anterior view of sternal plates, as in E<sub>1</sub>.

1, rib; 2, pectoral muscles; 3, sternal plate; 4, heart; 5, sheet of flattened cells; 6, keel; 7, coracoid articulation; 8, anterior lateral process; 9, spina sterni; 10, corpus sterni; 11, fontanelle; 12, posterior lateral process.

so that they are vertical and parallel, converging somewhat toward the midline at the anterior end. Posteriorly, they have fused with the first five sternal ribs, and reach beyond the fifth. Chondrification has begun in an anterolateral sector. At the end of the eighth day (cf. Fig. 369-D, D<sub>1</sub>, and D<sub>2</sub>) in the parakeet (*Fell, 1939*), fusion of the halves begins at the anterior extremity, and they are drawn from a vertical position into a concave form



by progressive ventral fusion. Union of the plates takes place on the ninth day of incubation for the chick and for the duck, *Anas platyrhynchos* (Hommes, 1924). In the 8-day parakeet embryo, the anterior end of the sternum has extended beyond the coracosternal articulation, and connection is being made caudally with the sixth sternal rib. Chondrification is complete in the chick on the eleventh day (Hommes, 1924), and in the parakeet on the ninth day, except for the growing anterior, posterior, and ventral margins. Between the ninth and tenth day for the parakeet (Fell, 1939), the posterior lateral processes appear as procartilage (cf. Fig. 369-E<sub>1</sub> and E<sub>2</sub>); they have been described as mesenchymal aggregates on the seventh day in the chick (Hommes, 1924). As the lateral ends of the plates move ventrally, the body of the sternum becomes planar rather than concave; at the medial line of fusion the keel begins to take shape as a vertical, ventral projection (Fell, 1939).

**Formation of the keel.** Union of the sternal plates on the ninth day of incubation gives rise to part of the anterior portion of the keel in the parakeet, *Melopsittacus undulatus* (Fell, 1939), as shown in Fig. 369-D; the cranial extremity of the structure at this time is represented by a median mass of procartilage from which will arise the anteriorly projecting spina sterni (Fig. 369-D<sub>1</sub>, D<sub>2</sub>, E<sub>1</sub>, and E<sub>2</sub>). Development of the keel takes place more posteriorly as fusion of the sternal plates continues in this direction.

Ventral extension of the keel is accomplished by downward migration into the midline of cells from the sternal perichondrium during the ninth and tenth days (Fell, 1939). In advance of this migration, degeneration of mesodermal tissue occurs in a narrow strip along the ventral midline of the embryo parakeet, *Melopsittacus undulatus*, fowl, *Gallus gallus*, and sparrow, *Passer domesticus* (Fell, 1939). The strip reaches caudally as far as the yolk stalk, and the greatest degeneration is seen in the area where the sternal plates are about to fuse. Even the ectoderm shows necrosis in a median strip, and is thickened, evidently as a result of contraction. Chondrification begins in the keel at about 10 days, in the anterior and dorsal portions. It reaches its maximum relative length on the eleventh or twelfth day (cf. Fig. 369-E, E<sub>1</sub>, and E<sub>2</sub>; Fell, 1939).

The studies of Fell (1939) on the parakeet (*Melopsittacus undulatus*) indicate that the downward movement and rotation of the sternal plates accompanies a general ventral migration of mesodermal tissue along the length of the body cavity.

Ossification of the sternum begins on the twenty-first day in the chick (Schinz and Zangerl, 1937).

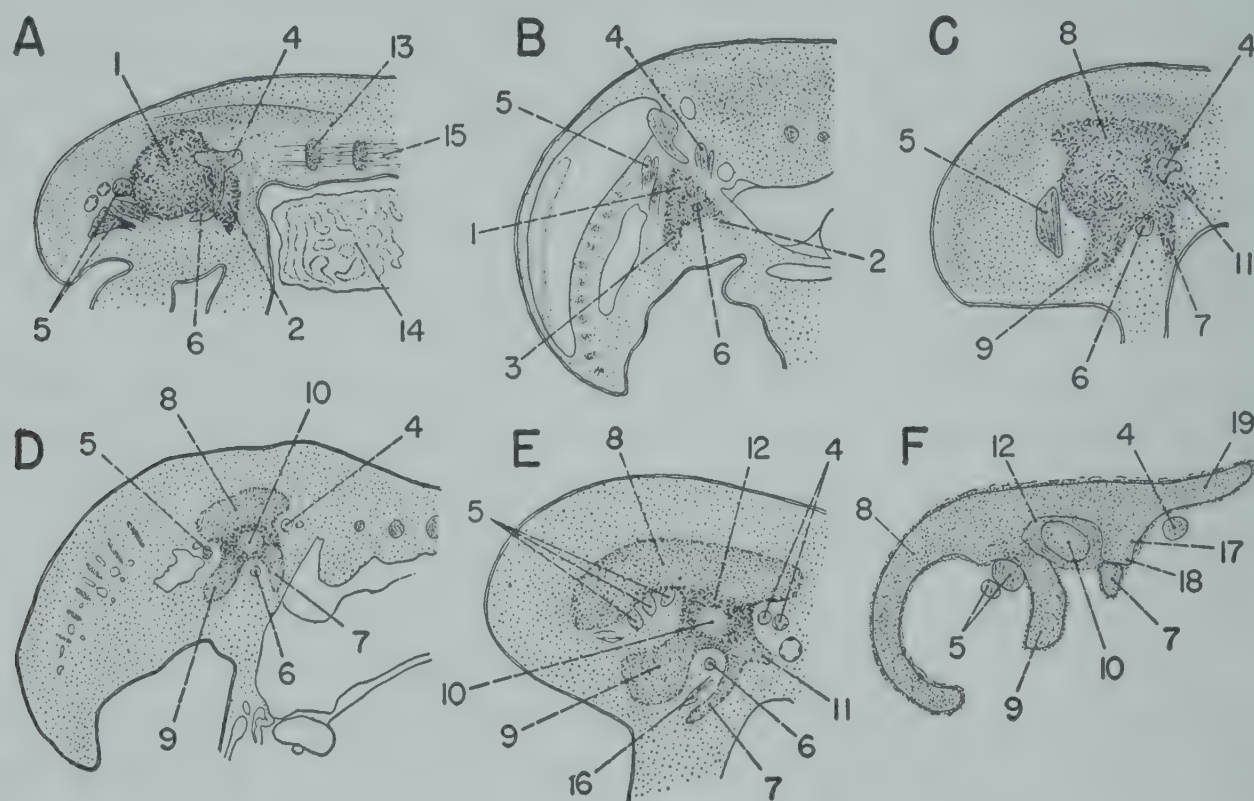
### *The Pelvic Girdle*

A single bone, the innominate or os coxae, provides support for the pelvic organs in birds. The innominate is derived from three embryologically



independent cartilages on each side: the dorsal ilium, the lateral ischium, and the ventral and anterior pubis.

The three elements of the pelvic girdle originate from a single mesenchymal condensation on the fourth day of the chick's incubation period (Bunge, 1880; Lebedinsky, 1913) or the fifth day as shown in Fig. 370-A (Johnson, 1883; Mehnert, 1887; Fell and Canti, 1934; Muratori and Franceschini, 1945). The girdle has been described by Johnson (1883) as two



**Fig. 370.** Early development of the avian pelvic girdle. (Redrawn with modifications A, C, and E, after Johnson, 1883; B, D, and F, after Mehnert, 1887.)

A, C, and E, diagrams of the pelvic condensation of the chick at 5.5, 6, and 6.5 days of incubation (limb bud lengths 3, 3.8, and 5 mm.); B, sagittal section through the girdle of the crested grebe (*Podiceps cristatus*) at a stage comparable to A (length of limb bud 4 mm.); D, similar section through the girdle of the gull (*Larus ridibundus*) at a stage comparable to C (limb bud 4 mm.), showing the individuality of the three cartilages; F, same section through the pelvis of the chick (*Gallus gallus*), at 6.5 days. All  $\times 10$ .

1, mesenchymal anlage of the pelvic girdle; 2, anterior ventral process of the pelvic anlage; 3, posterior ventral process of the pelvic anlage; 4, crural nerves; 5, ischiatic nerves; 6, obturator nerve; 7, pubis; 8, ilium; 9, ischium; 10, head of femur; 11, pectineal process; 12, acetabulum; 13, neural arch; 14, Wolffian body; 15, muscle plate; 16, muscles; 17, processus ilei acetabularis pubicus; 18, base of the spina iliaca; 19, pre-acetabular portion of the ilium.

short processes of the basal mesoderm of the limb bud; Mehnert (1887) and Lebedinsky (1913) found that the pelvis at this time consists of a dorsal plate of cells with its long axis parallel to the spinal column, representing the ilium, and two ventral processes, the anterior primordium of the pubis and a posterior process which will become the ischium. Evidently growth of the girdle condensation is rapid during its first day. It is completely continuous with the compact, cylindrical femoral condensation at



the region of the future acetabulum (Johnson, 1883; Mehnert, 1887; Lebedinsky, 1913; Fell and Canti, 1934).

Early chondrogenic centers for the pelvic bones are present on the sixth day in the chick. According to Johnson (1883) no separation was found between the anlage of the three bones, but most workers have asserted that the pubis has a distinct center (Bunge, 1880; Mehnert, 1887; Lebedinsky, 1913). A preacetabular process of the ilium (Fig. 370-F) is first formed as mesenchyme at this time; it has an anterior extension, the spina iliaca, which Johnson (1883) erroneously identified as a prepubis (Mehnert, 1887). The process, including the spina, is chondrified on the eighth day as an integral part of the ilium (Mehnert, 1887).

Connection between the cartilaginous anlagen is not a typical avian condition, according to Mehnert (1887). In a large group of birds, as in the *Reptilia*, all three of the early chondral elements of the pelvic girdle are separate. Chondrogenesis may begin either in the center of the mesenchymal aggregation, as in the mew and black-headed gulls, *Larus canus* and *Larus ridibundus*, the tern, *Sterna hirundo*, and the grebe, *Podiceps* sp., or in the three separate acetabular processes in the oyster catcher, *Haematopus ostralegus*, the duck, *Anas platyrhynchos*, the crow, *Corvus cornix*, and the rook, *Corvus frugilegus* (Mehnert, 1887).

In the chick, separation of the femur from the pelvic structures by a zone of densely packed mesenchyme begins at about the fifth day (O'Rahilly and Gardner, 1956). Deposition of cartilage continues, being always in a less advanced state in the girdle than in the femur (Johnson, 1883). The pubis and ischium have begun to grow in a posterior direction, and during the following days they assume their adult relation with long axes parallel to the long axis of the ilium and to the spinal column (cf. Fig. 370-C and E; Fig. 371). The increase in length of the pelvic elements is presented in the following table, from the data of Lebedinsky (1913).

Age of Chick (days)	Length of Pelvic Elements		
	Ilium (mm.)	Pubis (mm.)	Ischium (mm.)
7	2.9	1.5	1.3
8	3.8	2.2	1.9
9	6.0	4.1	3.0
10	7.5		
12	10.0		
13	11.0		
15	14.0		
19	20.5		

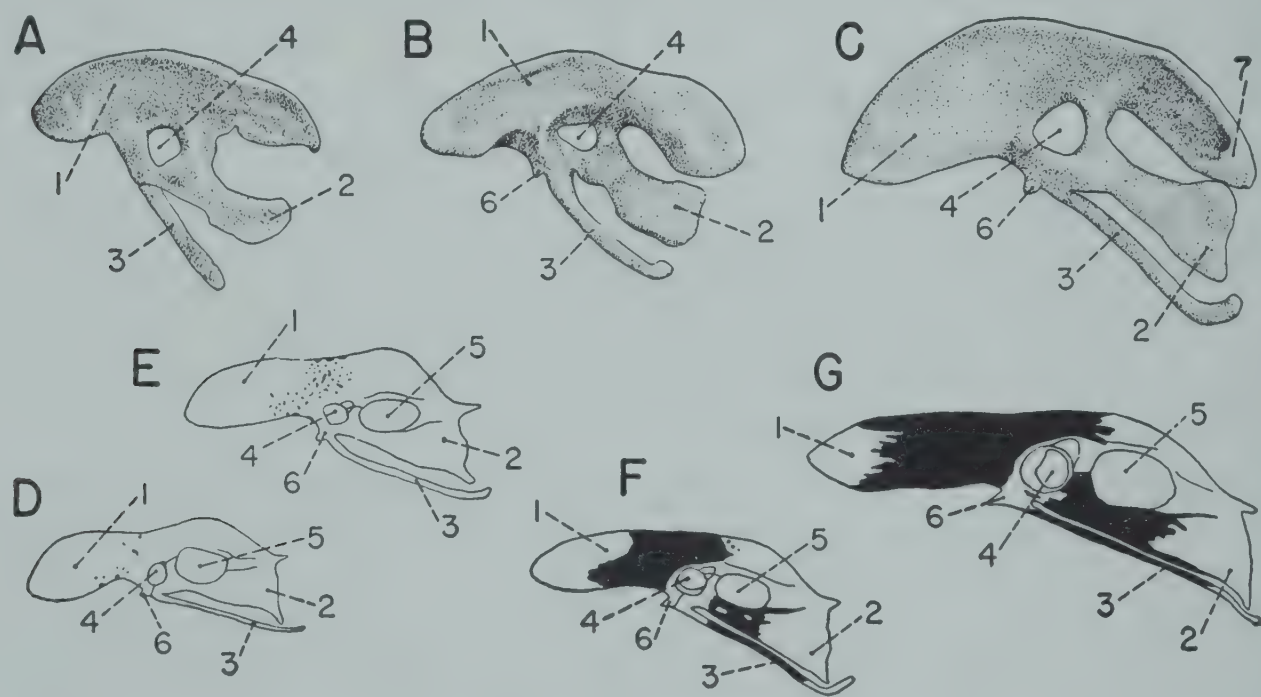
The condition of the pelvic bone of most ratite birds corresponds to an early embryonic stage of carinates with respect to the angle which the long axes of the pubis and ischium form with the ilium (cf. Fig. 371).



Lebedinsky's (1913) comparison of chick embryos of different ages, the primitive bird (*Archaeopteryx*), and two modern adult ratites is shown in the accompanying table.

Age of Chick Embryo (days)	Angle between Pubis and Ilium	Adult Form Having Corresponding Angle
7.0	45°	Primitive bird ( <i>Archaeopteryx</i> )
7.3	35°	Kiwi ( <i>Apteryx oweni</i> )
8.0	30°	Ostrich ( <i>Struthio camelus</i> )

The ischium and ilium are in contact at their caudal ends on the ninth day in the chick, forming the posterior boundary of the ischiadic foramen; they are fused on the tenth day.



**Fig. 371.** Development of the pelvis (innominate bone) of the chick from the seventh to eighteenth days of embryonic life, showing the change in position of the pubic and ischial cartilages and the beginning of ossification. (Redrawn with modifications after Lebedinsky, 1913.)

A to G, lateral aspect of left pelvic bone at 7, 7.5, 8, 12, 13, 15, and 18 days of incubation. Bone is shown in black (A to C,  $\times 12$ ; D to G,  $\times 2$ ).  
1, ilium; 2, ischium; 3, pubis; 4, acetabulum; 5, foramen ischiadicum; 6, pectineal process; 7, ischiosacral crista.

The ilium begins to ossify on the twelfth day of the chick's incubation period, and a perichondral deposit of bone is present in the center of the pubis on the following day (Lebedinsky, 1913). By the fifteenth day, the ischium is also ossifying beneath the foramen ischiadicum. The condition of the innominate bone 3 days before hatching is shown in Fig. 371. Fusion of the pubis with the ventral margin of the ischium has not yet taken place. The acetabular region is cartilaginous at hatching, and union of the ilium with the sacral vertebrae occurs during the postembryonic period by ossification of the iliosacral ligaments (Chamberlain, 1943).



*Development of the Hindlimb*

The limb skeleton is formed from a condensation which has the shape of a letter Y in the 5-day chick (*Fell and Canti, 1934*). The distal arms of the Y will give rise to the tibia and fibula, and are more diffuse at this time than the proximal femoral condensation. Elongation of the cellular rudiments is rapid, and the three major leg components are prochondral by the sixth day in the chick (*Johnson, 1883; Fell and Canti, 1934*) and the duck, *Anas platyrhynchos* (*Sieglbauer, 1911*). Membranous precursors of the tarsus and metatarsals are present in the chick on the sixth day (*Johnson, 1883*). Separation of the femur from the pelvis by a dense cellular interzonal region at the presumptive acetabular articulation begins on the fifth day in the chick (*O'Rahilly and Gardner, 1956*).

**Differentiation of the femur.** Chondrification begins in the diaphysial region at 5.5 days (*Muratori and Franceschini, 1945*), and is in an advanced state in the thigh bone of the chick incubated 6 days; the first layer of periosteal bone is forming at this time (*Fell, 1939*). The distal condyles and articular groove for the head of the fibula are present (*O'Rahilly and Gardner, 1956*). On the eighth day (*Fell, 1939*), excavation of the marrow cavity begins. The femur is the stoutest element during the chondral stage, but its growth rate is second to that of the tibia. The data of *Stepanova (1926)*, *Landauer (1939)*, and *Haardick (1941)* demonstrate the growth of the components of the hindlimb, as shown in Appendix Table VIII.

**Development of the patella.** The patella and its ligament are formed from a common mesenchymal mass on the anterior side of the region of junction between the femur and tibia. The ligament appears first; on the fifth day in the chick according to *O'Rahilly and Gardner (1956)*, there is a distinct strip of undifferentiated cells along the anterior surface of the cell mass, reaching distally toward the chondrifying tibia. Collagenous fibers can be found in this strip at 6.5 days of development. Late in the seventh day, the patella can be distinguished as a condensation of cells deep in the ligament at the distal end of the femur; its boundary is still diffuse due to the absence of a perichondrium. The primordium appears to be periarticular rather than intratendinous.

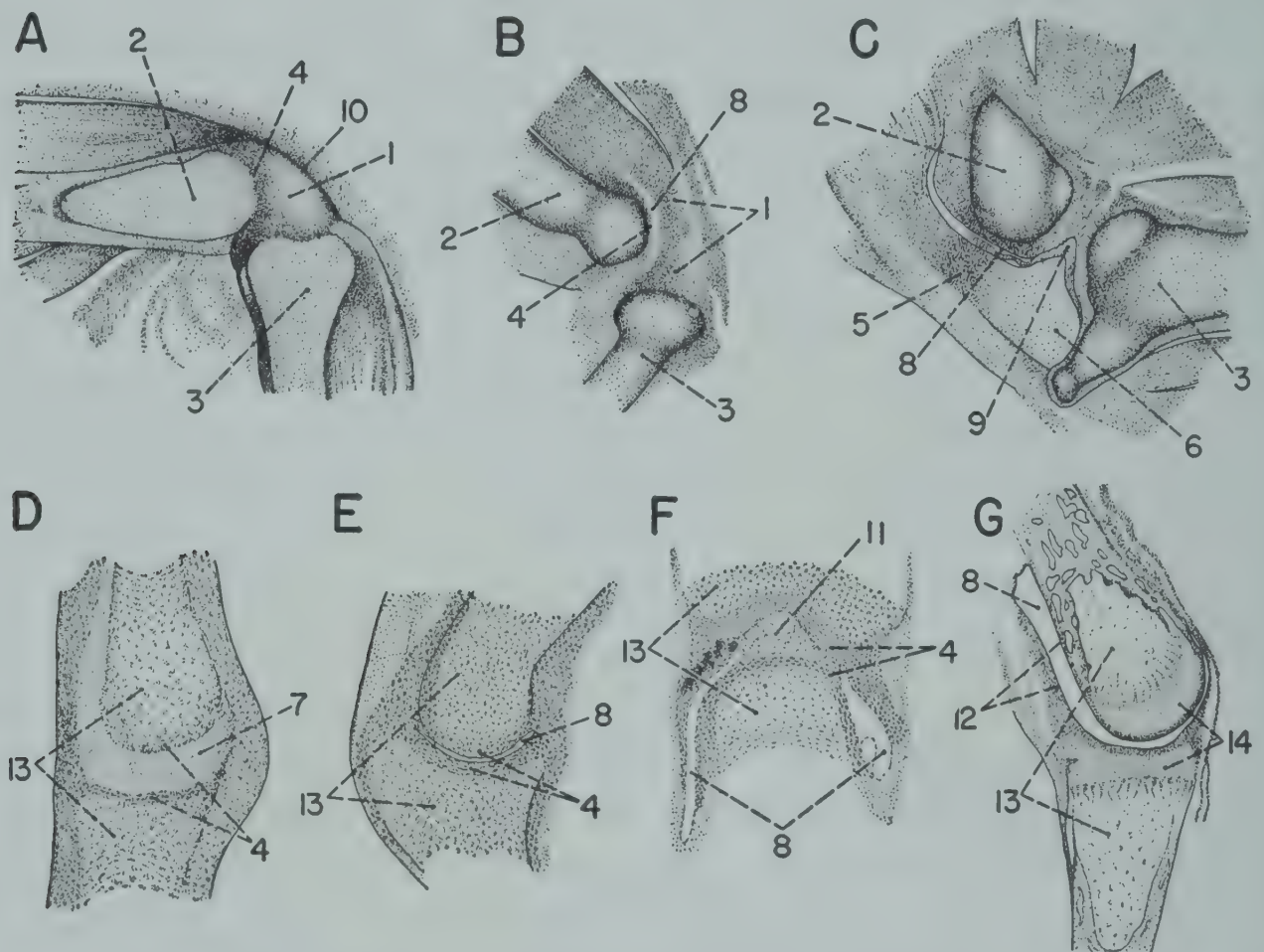
On the eighth day of the chick's incubation period, formation of the articular cavity begins in the interzonal tissue between the femur and the patellar disc (Fig. 372). Cavitation proceeds from the periphery to the center of the interzone (*Hepburn, 1889*); it takes place also between femur and fibula at this time (*O'Rahilly and Gardner, 1956*).

By the ninth day, according to *Kaczander (1887)*, the cells composing the patella are small, round, and tightly packed, but show as yet no intercellular matrix. Chondrification begins on the tenth day (*O'Rahilly and Gardner, 1956*). Intercellular material appears at the same time in the



ventral portion of the patellar mass, and it becomes separated from the chondrifying disc. Much of this tissue disintegrates, and a large deposit of adipose tissue has been formed in its place by the eighteenth day. At hatching time, the part of the patella which projects into the articular cavity is covered with connective tissue cells derived from the intermediate zone.

The patella is ossified 11 weeks after hatching (*Niven, 1933*).



**Fig. 372.** Successive stages in the development of the patella and of the diarthrodial joints of the chick embryo. (Redrawn with modifications A to C, after Kaczander, 1887; D to G, after Hepburn, 1889.)

A, knee joint during the ninth incubation day ( $\times 10$ ); B, the same, later in the ninth incubation day ( $\times 20$ ); C, the same, during the eleventh incubation day ( $\times 20$ ); D, longitudinal section of a phalangeal joint in the leg, during the ninth incubation day, showing the interzonal mesenchyme ( $\times 30$ ); E, longitudinal section of a phalangeal joint in the wing at the end of the ninth incubation day, showing the joint cavity ( $\times 30$ ); F, the same, during the middle of the second week of incubation, showing an interarticular ligament ( $\times 30$ ); G, longitudinal section of the long claw (middle digit) at the end of the nineteenth incubation day ( $\times 8$ ).

1, mesenchymal anlage of patella and fat cushion; 2, femur; 3, tibia; 4, precartilage; 5, patella; 6, fat cushion; 7, mesenchyme of interzonal disc; 8, joint cavity; 9, connective tissue; 10, ligamentum patella propria; 11, interarticular ligament; 12, lining of the synovial cavity; 13, phalanges; 14, epiphysis.

**Differentiation of the tibia and the fibula.** In the chick (*Johnson, 1883*) at 5 days and the duck (*Anas platyrhynchos*) at 6 days (*Sieglbauer, 1911*), the prochondral condensations of the tibia and the fibula are still connected to the femur and the metatarsal condensation. The process of cartilage



formation is always more advanced in the fibula than in the tibia (*Fell and Canti, 1934*). By the sixth day, these structures in the chick are distinct from each other and well chondrified. A comparable stage of differentiation is found in the ostrich (*Struthio camelus*) at 10 days (*Broom, 1906*), and in the 15-day emu, *Dromaeus novae-hollandiae* (*Lutz, 1942*). On the seventh day of incubation of the alpine swift (*Apus melba*) connection between the distal end of the fibula and the tarsus has become membranous, and only the tibia reaches the tarsal plate (*Zehnter, 1890*).

Early ossification centers appear in the tibia and fibula on the sixth day of incubation (*O'Rahilly and Gardner, 1956*). The cross section of the fibula is always less than that of the tibia, and this difference becomes more marked in further development. This bone ossifies mainly at its proximal end, tapering distally to a thin splint. It articulates with the tibia at the proximal end and also along the shaft.

**Differentiation of the tarsus.** The embryonic elements of the avian foot are somewhat obscure. The tarsus is composed of a proximal plate, which later fuses with the distal end of the tibia, and a distal plate, which becomes the proximal end of the metatarsus. The proximal row of tarsals consists in general of a large, probably compound fibulare, and a compound tritibiale. In the chick embryo, the tarsus appears at 5.5 days as a broad procartilagenous band continuous with adjacent limb elements (*Johnson, 1883*). On the sixth day the tibiale and fibulare can be distinguished as cartilage centers, each directly distal to its crural counterpart. *Holmgren (1933)* has observed that the tibiale of the weaverbird, *Ploceus* sp., has three distinct parts in its earliest embryonic stage. This author suggests that these represent the intermedium and centralia I and II. A separate intermedium appears between the tibiale and the fibulare in the chick of 6 days (*Johnson, 1883*), in the 10-day ostrich, *Struthio camelus* (*Broom, 1906*), and in the penguin, *Pygoscelis papua* (*Sieglbauer, 1911*). This element becomes the ascending process of the astragalus or ankle bone (see Fig. 373-D).

The fibulare is present as hyaline cartilage in the 6-day duck, *Anas platyrhynchos* (Fig. 373-A; *Sieglbauer, 1911*). *Holmgren (1933)* has noted that in the embryo of the gull, *Larus canus*, this element has a proximo-plantar part and a distal part, the latter being more strongly chondrified; he suggests that these represent the fibulare proper and centrale IV, respectively.

A pisiform cartilage has been identified by *Lutz (1942)* in the emu, *Dromaeus novae-hollandiae*, at 15 days of incubation.

Four distal tarsals were described by *Zehnter (1890)* for the alpine swift, *Apus melba*, but only two, three, and four have been distinguished in the 6-day duck, *Anas platyrhynchos* (*Sieglbauer, 1911*), and the 10-day ostrich, *Struthio camelus* (*Broom, 1906*). *Lutz (1942)*, however, found five



tarsal elements in the duck at 8 days (Fig. 373-B), and in the 15-day emu (*Dromaeus novae-hollandiae*).

All five metatarsal elements are present in the duck (*Anas platyrhynchos*) at 6 days (Fig. 373-A) as membranous condensations, but the fifth metatarsal develops only a very small chondral portion; it is transient, and fuses with the basal tarsals. A rudimentary fifth metatarsal was observed in the alpine swift (*Apus melba*) on the fifth day of incubation; it had disappeared by the seventh. The digital condition is very similar in the penguin (*Pygoscelis papua*). In the 10-day ostrich (*Struthio camelus*) embryo, however, the fifth metatarsal is more prominent than the first, although

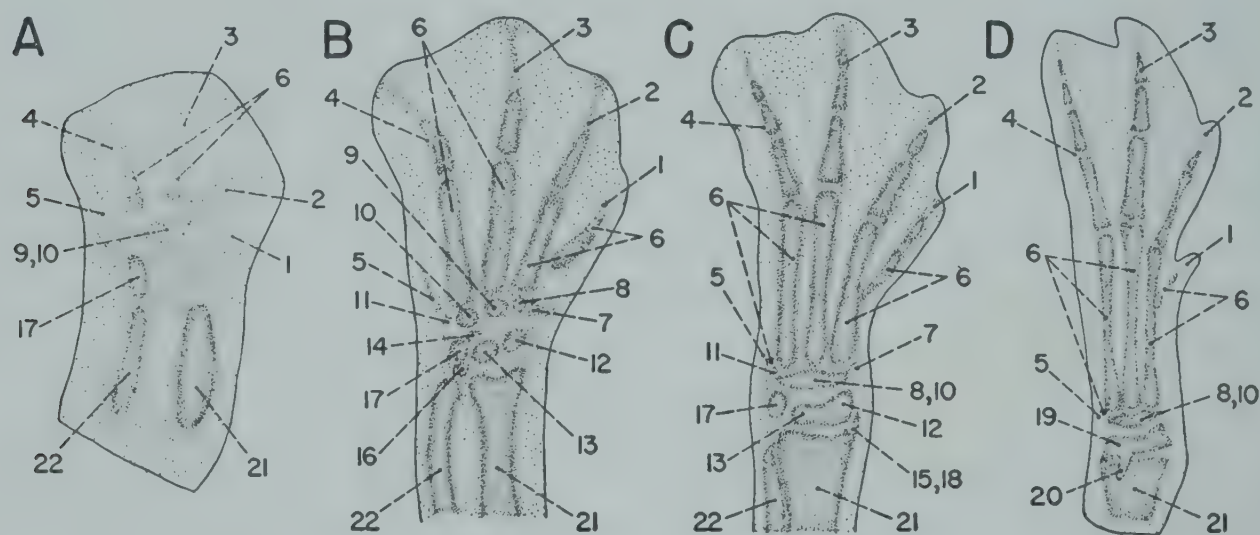


Fig. 373. Representative stages in cartilaginous development of the foot of the duck, *Anas platyrhynchos*. (Redrawn with modifications A and D, after Sieglbauer, 1911; B and C, after Lutz, 1942.)

A, dorsal aspect of the left foot at 6 days of incubation, from tracing reconstructions ( $\times 15$ ); B, C, D, same at 8.5, 9.5, and 10 days ( $\times 5$ ).

1-5, digits one through five; 6, metatarsals; 7-11, distal tarsals one through five; 12-14, central tarsals: 12, distal tibial centrale; 13, proximal tibial centrale; 14, proximal fibular centrale; 15, tibiale; 16, intermedium; 17, fibulare; 18, "prehallux"; 19, astragalus or ankle bone; 20, ascending process of astragalus; 21, tibia; 22, fibula.

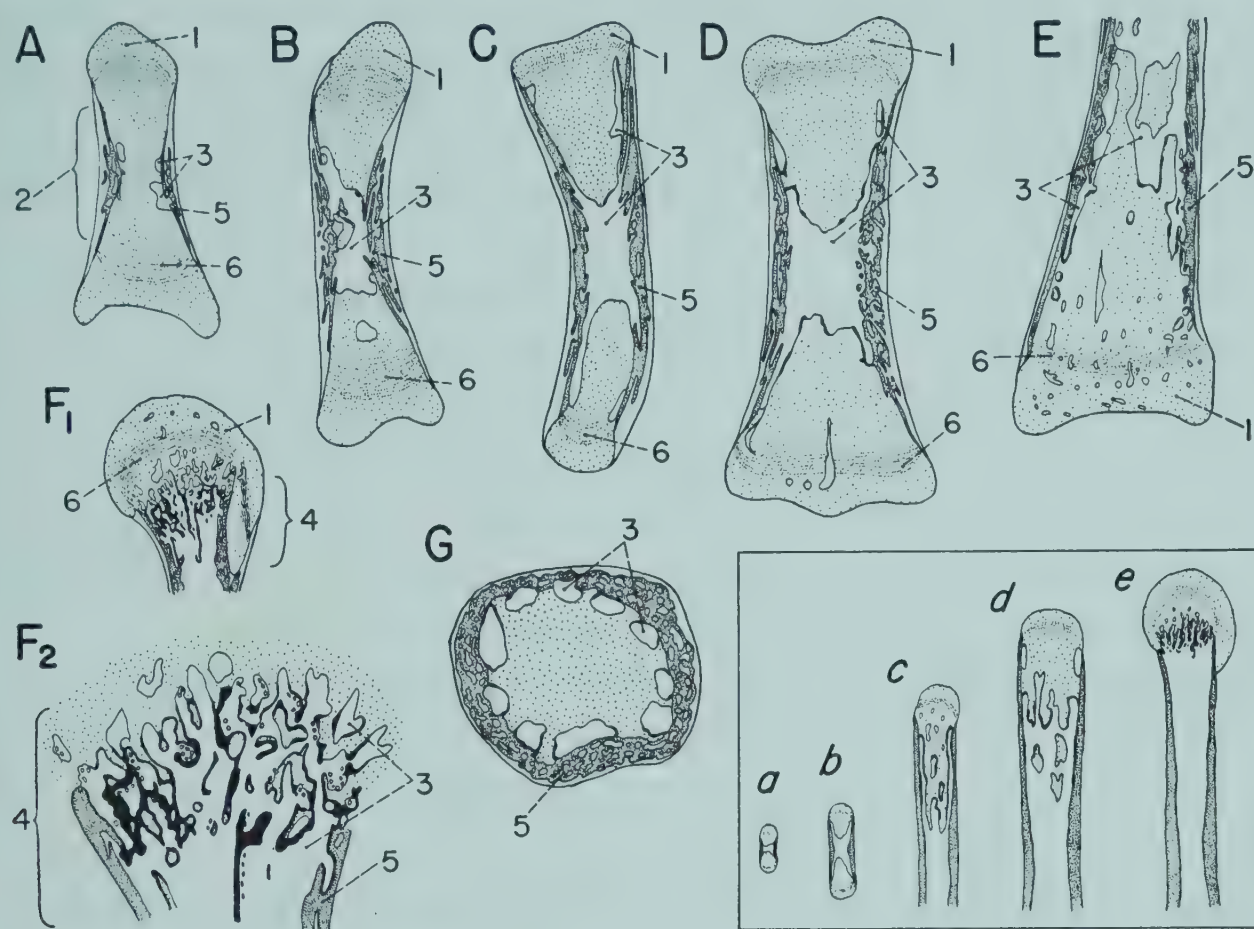
only the third and fourth digits will be functional in the adult. In the 9.5-day duck (Fig. 373-C), the first digit has diverged to a position of apposition (Lutz, 1942).

**Differentiation of the phalanges.** At the distal end of each metatarsal appears a mass of condensed precartilaginous tissue; the phalanges are produced by the lengthening and segmentation of this mass (Fig. 374). Chondrification is beginning in the proximal segments on the eighth day of the duck's (*Anas platyrhynchos*) development. The phalangeal formula is constant for each digit, increasing from two on the first to five on the fourth; it is not altered for the functional third to fourteenth digits in running birds.

**Formation of the diarthrodial joint.** During the early stages of chondrogenesis in the long bones of the limbs, the interzones between the rudi-



ments remain mesenchymal (cf. Fig. 372-D), although a trace of precartilaginous matrix can sometimes be found among the cells (*Johnson, 1883; Fell and Canti, 1934; O'Rahilly and Gardner, 1956*). This tissue gives rise to the lining of the synovial cavity (cf. Fig. 372-G), to ligaments connecting the bones, to articular cartilages covering the surfaces of the



**Fig. 374.** A semidiagrammatic representation of the process of cartilage erosion and the subsequent formation of bone in the phalanges and long bones in the hindlimb of the chick. (Redrawn with modifications after Lubosch, 1923.)

A, second phalanx of toe, from 10-day embryo ( $\times 10$ ); B, next to last phalanx, third toe, same stage ( $\times 10$ ); C, the same, 1 day after hatching ( $\times 5$ ); D, proximal phalanx of third toe, same stage ( $\times 5$ ); E, distal end of tibia, same stage ( $\times 5$ ); F<sub>1</sub>, proximal end of a metatarsal, from 14-day embryo ( $\times 5$ ); F<sub>2</sub>, detail of endochondral ossification ( $\times 30$ ); G, cross section through proximal end of a metatarsal, from 20-day embryo, showing peripheral spaces in cartilage lined with endochondral bone ( $\times 15$ ).

*Insert:* illustrates schematically the relative changes in proportion during the three stages in the process of ossification: *a*, stage 1, formation of the medullary cavities; *b* to *d*, stage 2, erosion of the calcified cartilage in the medullary cavities, which begins during the second half of incubation and continues for 14 days after hatching; *e*, stage 3, the formation of medullary bone.

1, epiphysis; 2, diaphysis; 3, marrow cavity; 4, zone of endochondral ossification; 5, perichondral ossification; 6, proliferative zone.

skeletal elements, and, in the case of the femorotibial articulation, to two fibrocartilages located with the joint, known as the medial and lateral menisci.

At 4.5 days (*O'Rahilly and Gardner, 1956*), these interzones have a high cell density and are homogeneous in structure, without orientation



of cells or nuclei. Arrangement of the nuclei transverse to the long axis of the bones has been observed in the knee interzone at 5.5 days. Subsequently the cells become flattened along the lines of the future articular surfaces. A cleft appears in the peripheral part of the femorotibial interzone, proximal to the primordium of either menisci, at 8 days; between 8 and 10 days of incubation, it proceeds toward the center of the zone, widens considerably, and becomes continuous with the space between femur and patella (cf. Fig. 372-A, B, and C). Cavitation of the hip joint was described by Muratori and Franceschini (1945) on the eighth day. The wing articulations develop slightly later than those in the leg (O'Rahilly and Gardner, 1956).

Very early cartilage was noted by O'Rahilly and Gardner (1956) in the articular cartilages and menisci at 11 days. In other joints lacking menisci, such as the interphalangeal articulations, cartilage formation in the articular surfaces may precede cavitation. According to Hepburn (1889), in the digits of the chick manus the distal margins of the interzones undergo an abrupt transition to cartilage at 9 days; formation of the cavity then begins at the periphery of the central zone.

A capillary network underlying the synovial tissue is present in some areas at 11 days (O'Rahilly and Gardner, 1956). At the time of hatching, the articular surfaces and menisci are composed for the most part of dense fibrous tissue or early fibrocartilage.

Fell and Canti (1934) have found that orientation of mesenchymal cells in the interzone along the future articular surfaces will take place in tissue culture, and is probably brought about by pressure from the rapidly growing long bones. Later stages of joint formation evidently require some external influences.

## THE INTEGUMENT

The integument or skin is composed of the epidermis and dermis, and their derivatives. Of the latter the keratinized structures are the feathers, beak, egg tooth, beak cushion, scales, spurs, and claws; the vascularized structures are the comb, wattles, and toe pads; and the glandular is the uropygial gland.

### *The Epidermis*

The primitive form of the skin arises from the ectoderm and includes the fibrous basal membrane which develops in the chick between the second and fourth day (Snessarew, 1934) of incubation. Its loose fibers become impregnated with an elastic collagenous substance. At early stages the epidermis is a single layer of cells. On the second day of incubation it starts to separate into an outer layer and an inner mucous layer (Gardiner,



1885; Kingsbury, Allen, and Rotheram, 1953). Early on the third day the cells covering the ventral surface of the embryonic head lose their stellate form and consist of two irregular layers, a superficial layer of squamous cells or epitrichium and a compact layer of cylindrical cells next to the rapidly proliferating mesenchyme. On the fifth day this inner mucous layer has large cuboidal cells with one or more dark nucleoli and begins to form a horn layer in the epitrichium. The cell walls are thicker at their base than at the upper surface where they are sometimes indistinct. The next day the cells divide off from the mucous layer (Jeffries, 1884; Gardiner, 1885). In the developing embryo the outer cells are pushed wide apart and the underlying cells of the mucous layer push into the spaces left.

Specialized development of the epidermis of the various skin derivatives is considered in subsequent discussions of those structures. In general, the epidermis remains thin where it expands to cover the enlarging embryo. As the epidermis thickens by mitoses of basal cells in restricted areas (Kingsbury, Allen, and Rotheram, 1953), the cells of the intermediate layers are polyhedral and the superficial cells flattened. By thirteen days the cuboidal layer of cells proliferates, forming a stratified squamous epithelium, and the outer layers become more cornified. By the fourteenth day (Jeffries, 1884) the epitrichial cells are still irregular in shape and show large patches of intercellular substance between them.

The germinative layer, also known as the Malpighian layer, rete mucosum, or stratum germinativum, adjacent to the dermo-epidermal boundary, progressively adds to the cornified layers or stratum corneum. Where horn is to develop, as in the beak region, the stratum corneum is thicker and the horn layer develops faster than in the skin of the back and the rest of the head. The epitrichium is shed with the outer cornified cells as the epidermis assumes its final form.

### *The Dermis*

The dermis, cutis, or corium, arises from the outer cells of the somatic mesoderm and in part from the somites as a group of mesenchymal cells which gives rise about the twelfth day to connective tissue, and later, capillaries and nerves. Skin derivatives originate as local condensations within the dermis which initiate specialized development in the epidermis above it. Muscles, associated with feather development, arise in the dermis.

The dermis of the limbs, flank, and ventral surface of the body is derived from the mesoderm of the somatopleure. The dermatome of the somite becomes associated with the ectoderm (Rawles, 1955) in contributing to the formation of the dermis of the dorsal and dorsolateral body regions. The mesenchyme of the head is of neural crest origin and the head skin very likely is derived from it.

The properties of the mesoderm exercise a primary controlling influence



in the realization of regionally specific characteristics in the overlying ectoderm (Cairns and Saunders, 1954) such as pigmentation, formation of feather papillae, and the capacity of the skin to respond to hormones (Rawles, 1955). At first the dermis is a netlike, lamellar tissue forming an upper layer of the corium. This layer, as well as that of the developing feather papillae (Snessarew, 1934), undergoes modification to become a dense-celled, fibrous layer, which is the transitional form of the connective tissue with its reticular stroma. A lower, deep layer of the corium consists of pars terminalis and pars intermedia. The subcutis layer, also consisting at first mostly of netlike lamellar tissue of the mesenchyme, gives rise to loose connective and fatty tissue.

The dermis continues to develop slowly with the ingrowth of vessels and nerves and other undifferentiated cells being transformed into connective tissue and dermal muscles.

### Keratinized Structures

The process of keratinization occurs in feathers, beak, scales, spurs, and claws. As cells move out from the stratum germinativum to the stratum corneum, they acquire a horny exoplasmic membrane, the bridges become short and stiff, the cytoplasm and the nucleus are dry, and the nucleus disappears in the outermost cells. It has been noted that metachromasis and a basophilic cytoplasm are associated with the process. Also, an interaction of epithelium and mesenchyme seems to be involved. Cytoplasmic fibrils or tonofibrils have a function in the cornification process (Kingsbury, Allen, and Rotheram, 1953). They provide a structural continuity between the nonliving corneum and the living germinativum (Giroud and Leblond, 1951). The fibrils may be visible evidence of the synthesis of keratin, but whether they are intermediates or inducing substances in the process of keratinization is not known (Kingsbury, Allen, and Rotheram, 1953).

### Feathers

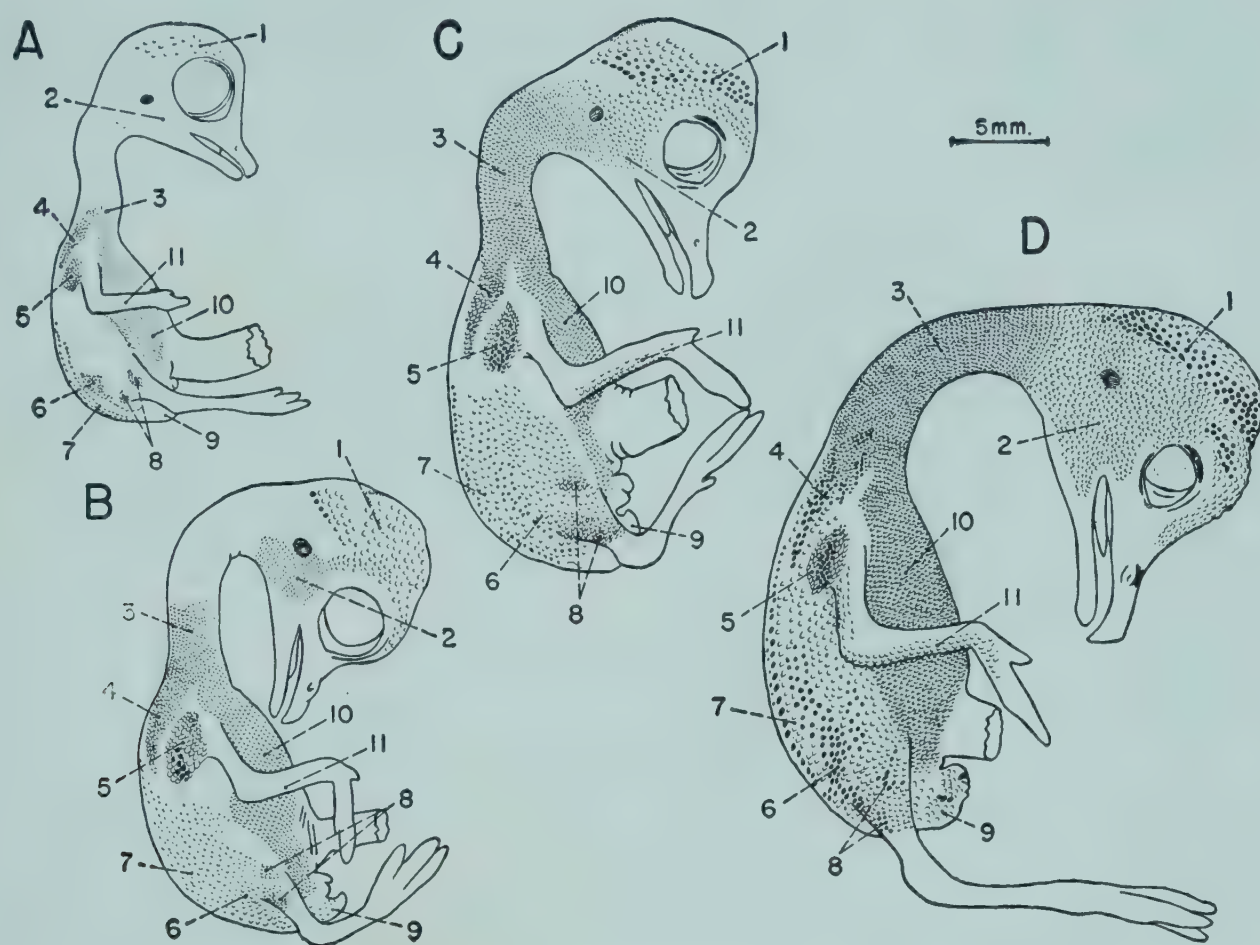
In the adult bird, feathers have a characteristic morphology of the tract in which they are located (Fig. 375). There are approximately eleven different tracts in the chicken (Holmes, 1935). Graded variations in shape, size, and pigmentation pattern occur also within tracts (Fraps and Juhn, 1936; Willier and Rawles, 1944a, 1944b).

The morphology of the thigh tract is determined very early in the embryonic period; in the chick at 3 to 4 days, before the appearance of feather precursors, transplantation of a segment of mesoderm from the leg to the wing results in a normal wing bearing typical leg feathers (Saunders, 1948; Cairns and Saunders, 1954). The dorsoventral axes of the feathers in the tract are also determined at this time (Saunders, 1948; Cairns and Saunders, 1954).



The morphological unit of the feather is the barb. The dorsal barbs are united to form the rachis or shaft; a smaller number of barbs fuse ventrally, giving rise to a hyporachis, and the resulting ventral structure is known as the aftershaft. Barbs bear lateral extensions, the barbules, which may have small projections with or without hooks called barbicels.

The arrangement of these basic structures in the down of the nestling and adult differs from that in the definitive feather. Down has a slight rachis, probably representing two barbs along most of its length, but joined



**Fig. 375.** Development of feather tracts in the crested grebe (*Podiceps cristatus*) embryo. (Redrawn with modifications after Portmann and Gerber, 1935.)

A, at 8 to 9 days' incubation; B, at 13 days; C, at 14 days; D, at 15 days. All in scale.

1, capital tract; 2, auricular tract; 3, cervical tract; 4, anterior dorsal tract; 5, humeral tract; 6, femoral tract; 7, posterior dorsal tract; 8, crural tract; 9, caudal tract; 10, ventral tract; 11, alar tract.

by other barbs proximally; the hyporachis is also short. Development of the vanes varies among avian species; the rachis and hyporachis of duckling (*Anas platyrhynchos*) down are much more pronounced than those of the chick (cf. Fig. 377 and 378). Barbules are borne on the proximal two thirds of the barbs. They are smooth and have no hooked barbicels, so that adjacent barbules are not held together.

The prototype of the adult feather has very many short barbs most of which are closely joined to the strongly keratinized rachis. Beneath the proximal part of the feather, there is an aftershaft of identical morphology, varying in size among the different tracts, but being always a mirror image of the feather. As the barbs of these structures lie closely adjacent, their



barbules intermesh, and are held together by many hooked barbicels, giving the feather its smooth surface. A small number of barbs at the base of the feather are not so connected, and these provide a downy layer.

*The down feather.* At 5 days of incubation in the chick (*Holmes, 1935*), a single ridge of condensed mesenchyme can be found in the dermis of the shoulder, thigh, and breast, running parallel to the long axis of the body in each case (Fig. 376). The ridge soon breaks up into a series of hillocks, which are the primordia of the feather papillae. The first row of feather germs is found in the duck (*Anas platyrhynchos*) at 8 days in both the spinal and femoral tracts (*Hosker, 1936*). Condensations appear in the wing of the chick on the sixth day. The cuboidal cells of the Malpighian

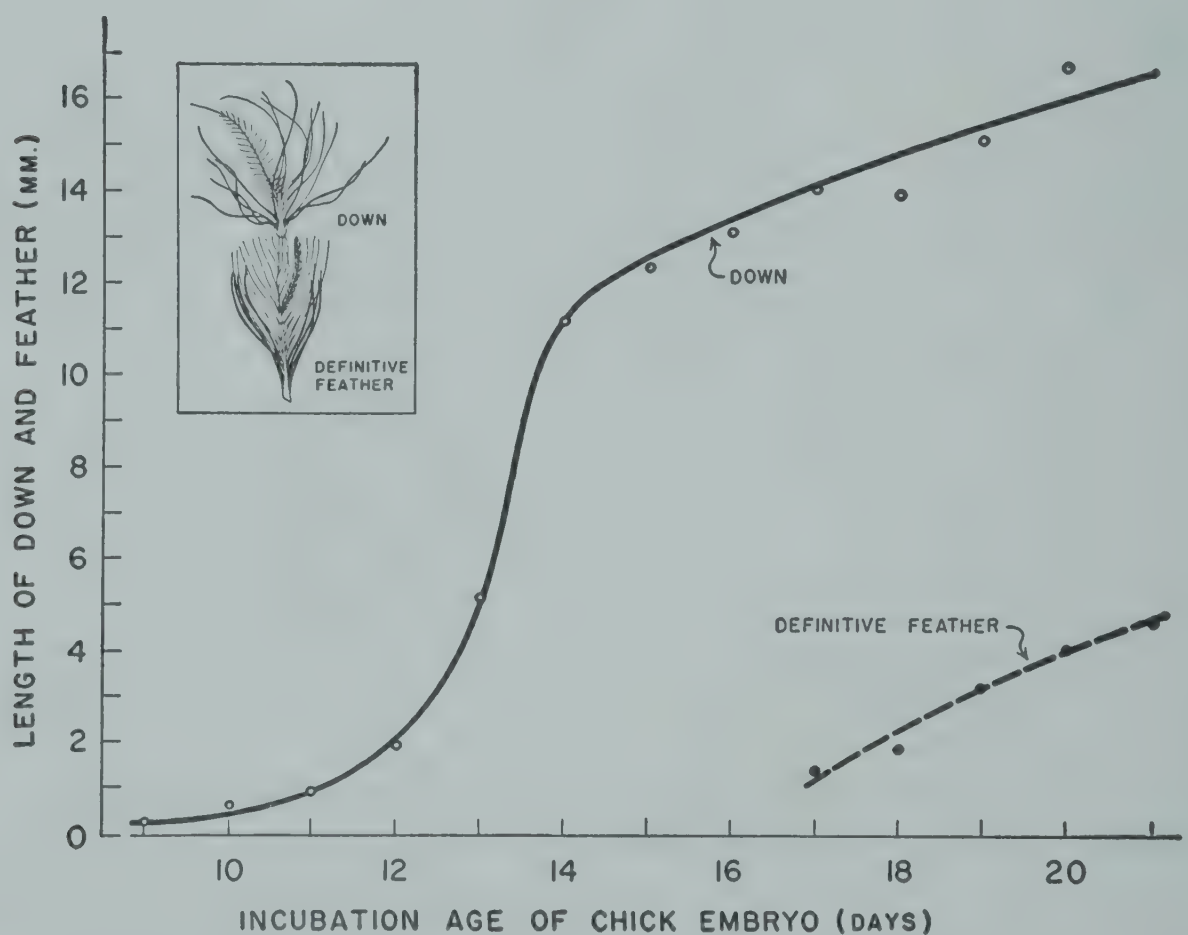


Fig. 376. Daily growth in length of down and definitive feather of the developing chick. (Plotted after data of Watterson, 1942.)

layer of the epidermis lengthen into a columnar form (*Davies, 1889; Hosker, 1936; Watterson, 1942*). In the following hours new ridges appear on either side of the initial one, and separate into condensations opposite the regions of low cell density in the first row. Succeeding primordia are also laid down in alternate rows in this manner. At 7.5 days, two rows of dermal aggregations are found parallel to the outer margin of the antebrachium (*Watterson, 1942*); the posterior row will give rise to the secondary flight feathers, and the anterior row to the major coverts (*Holmes, 1935*). In the regions of concentration of dermal cells, the layer of elongated epidermal cells now begins to divide, forming an intermediate layer beneath the epitrichium which proliferates to furnish most of the feather tissue (*Davies, 1889*). The



remaining cell stratum which limits the epidermal portion of the feather germ on the inner side is known from the shape of cells as the stratum cylindricum. Components of this layer may divide again to add a small number of intermediate cells (Hosker, 1936). According to Hosker (1936), some of the epitrichium in the duckling (*Anas platyrhynchos*) may also divide (Fig. 377-A), the daughter cells migrating medially into the inter-

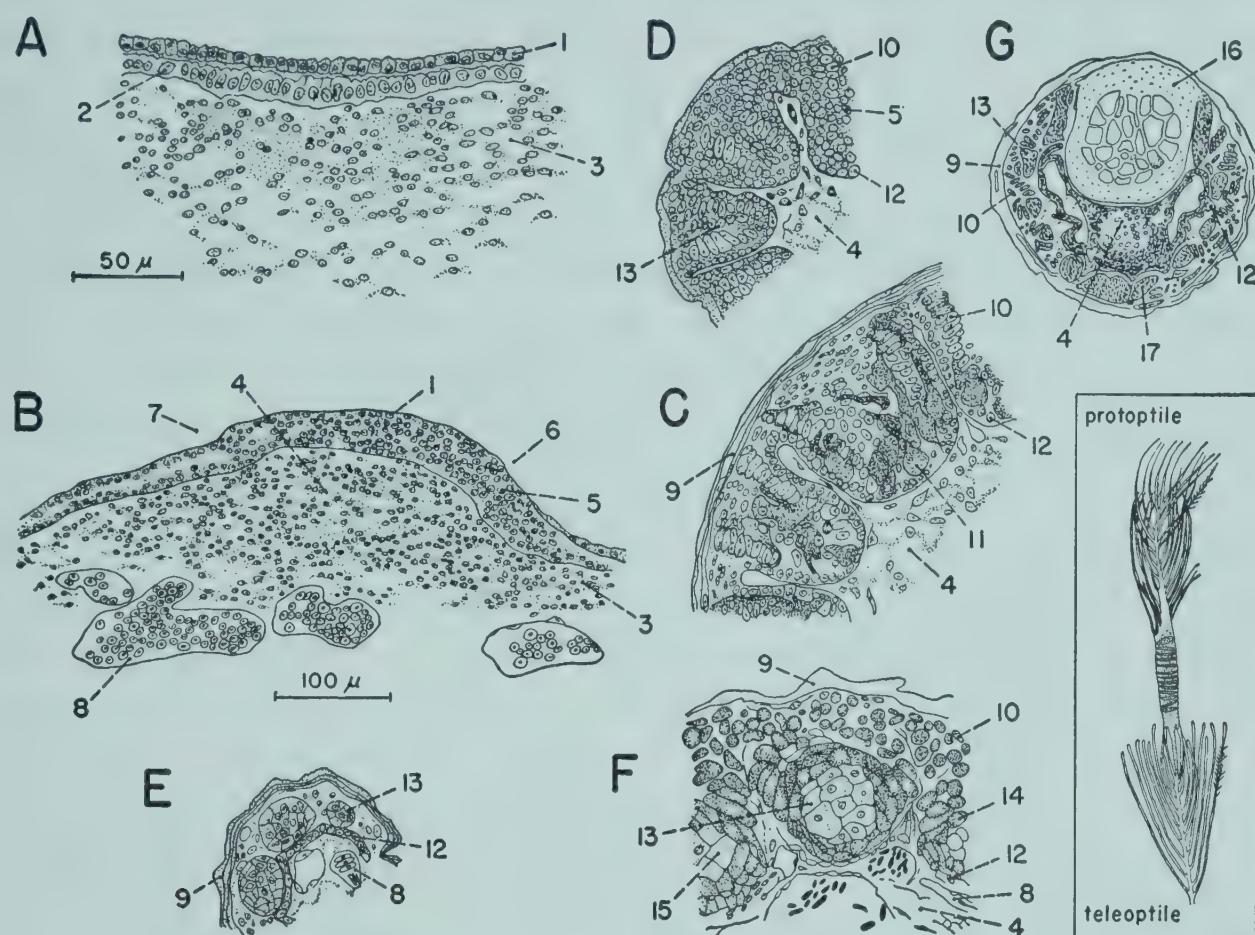


Fig. 377. Development of down feathers in the duck, *Anas platyrhynchos*, embryo. (Redrawn with modifications after Hosker, 1936.)

A, cross section of skin from the spinal tract of a 10-day embryo (in scale); B, the same, from an 11-day embryo, showing elevation of feather germ (in scale); C, detail of a cross section through the prepenna of a 15-day embryo ( $\times 450$ ); D, cross section through a feather filament of a 16-day embryo ( $\times 450$ ); E, cross section through the base of a feather filament from a 17-day embryo (in scale); F, cross section of a feather filament from an 18-day embryo ( $\times 900$ ); G, cross section through a protoptile from a 24-day embryo ( $\times 450$ ).

Insert: protoptile and teleoptile from the tail of a 6-week-old duckling.

1, epitrichial layer; 2, Malpighian layer; 3, dermis; 4, pulp; 5, intermediate cell; 6, anterior side of feather germ; 7, posterior side of same; 8, blood vessel; 9, sheath; 10, barbule; 11, pigment cell; 12, cylinder cell; 13, barb; 14, cortex; 15, medulla; 16, rachis; 17, hyporachis.

mediate mass. The stratum intermedium and the dermis proliferate rapidly, causing the feather germ to protrude from the surface of the skin; it is macroscopically visible by 9 days in the chick (Holmes, 1935; Watterson, 1942) and in the duckling, *Anas platyrhynchos* (Hosker, 1936). The melanophores in the epidermal thickening also divide rapidly (Watterson, 1942).



These papillae will give rise to the prepennae, which make up the down of the chick (*Hosker, 1936*). In the duck (*Anas platyrhynchos*) and goose (*Anser anser*) at this time, smaller papillae form a circle about the base of each larger papilla; these produce the preplumulae of the nestling, and later the plumulae or adult down. Even smaller papillae which appear later in these water birds produce prefiloplumae (*Gerber, 1939*), succeeded by filoplumae in the adult (*Hosker, 1936*).

The layers of the feather primordia grow more rapidly on the anterior side (Fig. 377-B) so that the germ is bent posteriorly until it is nearly parallel with the skin surface (*Davies, 1889; Hosker, 1936*). Proliferation then becomes restricted to the base of the primordium, and the distal two thirds begin to differentiate. Morphogenesis is always most advanced in the distal parts of the germ, and at any cross sectional level the dorsal side is found to be more completely developed (*Hosker, 1936; Watterson, 1942*).

On the tenth day the epidermis at the base of the primordium has begun to grow down into the dermis, forming the feather follicle (*Hosker, 1936*). The melanophores in the distal two thirds of the feather germ are becoming arranged in rows along the long axis of the feather primordium (*Watterson, 1942*). Intermediate cells become distributed in a corresponding series of ridges running parallel to the axis of the feather; by the end of the tenth day, a cross section through the distal portion of the germ (cf. Fig. 377-B) shows ten or eleven groups of intermediate cells, separated by the stratum cylindricum which reaches between them and is in contact with the epitrichium peripherally (*Hosker, 1936*). Each group will develop into a barb with its associated barbules. The dorsal ridges have the largest diameter. *Strong (1902)* considered the formation of ridges to be due to regrouping of the intermedial cells rather than to an invasion of the cell mass by the stratum cylindricum, and *Hosker (1936)* has drawn sections of the basal part of the germ in which ridge formation is complete before the cylinder layer changes its position. *Watterson (1942)* has observed that the intermediate cells increase greatly in volume during their reorientation (cf. Fig. 377-B and C).

On the eleventh day of incubation in the chick, the melanophores within a ridge take up a position along its inner margin. The medial portion of each ridge, the median plate, consists of undifferentiated intermediate cells without orientation, but the cells of the lateral margins have become somewhat elongated, and are aligned end to end in rows which are almost parallel to the axis of the feather, forming the barbules. A cross section of the feather germ at this time shows that the large melanophores, located at the pulp margin of the ridges, have processes leading to the most peripheral barbule cells. As these complete their keratinization, the processes of the pigment cells withdraw to more proximally located barbules (*Strong, 1902; Watterson, 1942*).



During the sixteenth and seventeenth days of incubation of the duck (*Anas platyrhynchos*) embryo, cells of the median plate move inward toward the apex of the ridge to form the barb (Hosker, 1936). The first of these become enlarged, vacuolated, and polygonal in shape (cf. Fig. 377-D and E); these become the medulla of the barb (Strong, 1902; Jones, 1907; Hosker, 1936). Other cells having the same origin become applied to the surface of the medulla, assuming an elliptical shape; on the eighteenth day

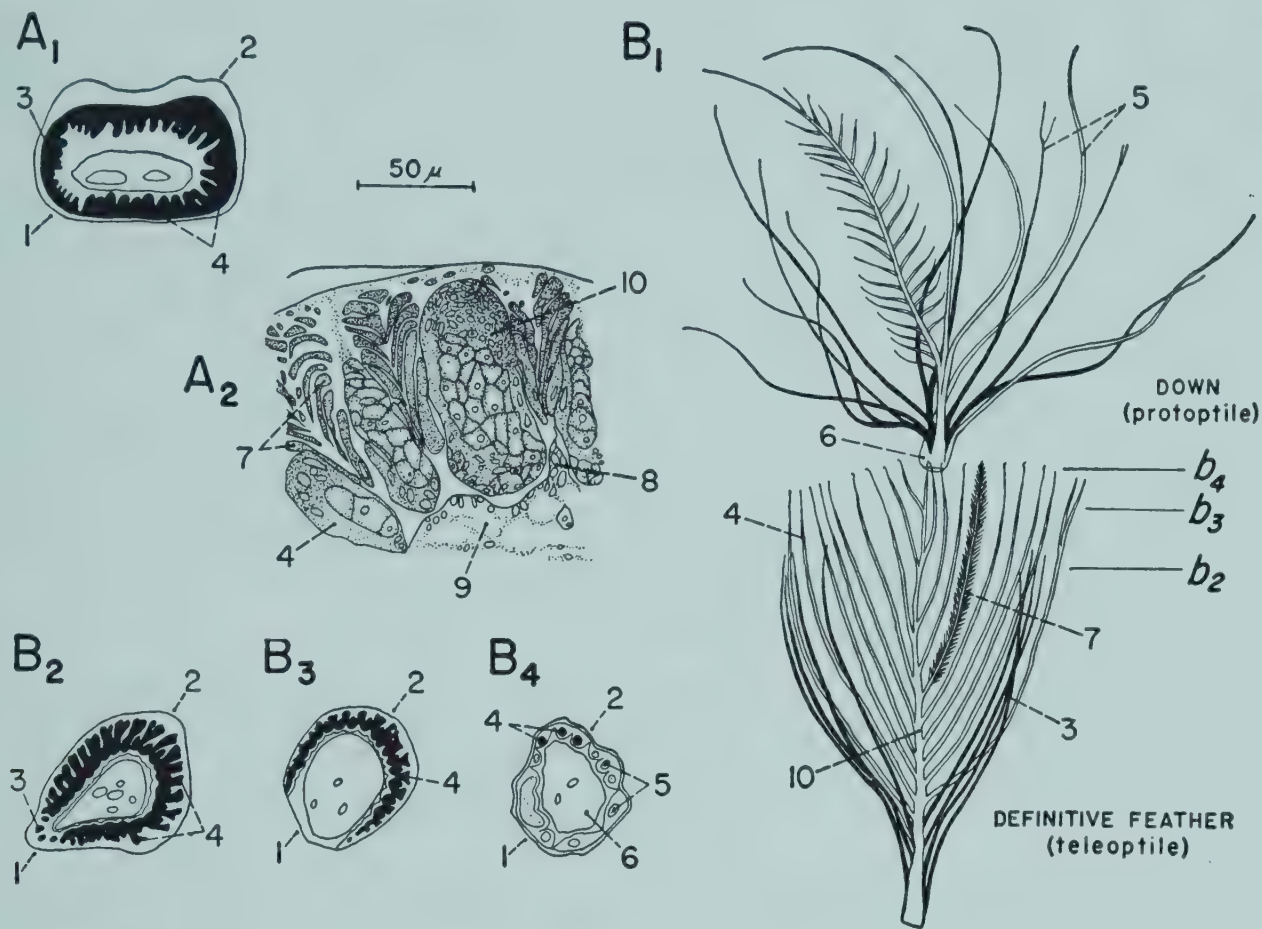


Fig. 378. Embryonic development of the first definitive feathers (teleoptiles) of the chick. (Redrawn with modifications after Hosker, 1936.)

A<sub>1</sub>, diagrammatic cross section near base of teleoptile from wing at 20 days' incubation; A<sub>2</sub>, detail of same, enlarged (in scale); B<sub>1</sub>, teleoptile with attached protoptile (down feather), from the wing of a day-old chick; B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>, diagrammatic cross sections of teleoptile, taken at levels indicated in B<sub>1</sub>: b<sub>2</sub> and b<sub>3</sub>, with sheath surrounding barbs intact; b<sub>4</sub>, showing the junction of the teleoptile with the protoptile.

1, ventral side of teleoptile; 2, dorsal side of same; 3, barbs of teleoptile aftershaft; 4, barbs distal to aftershaft; 5, barbs of protoptile; 6, base of protoptile; 7, barbules; 8, cylinder cell layer; 9, pulp; 10, rachis.

of the duck's (*Anas platyrhynchos*) incubation period they still have darkly-staining cytoplasm (Hosker, 1936; cf. Fig. 377-F). Growth of the barb extends the ridge toward the axis of the feather (Strong, 1902). The stratum cylindricum has been drawn out from its former compact arrangement into a string of cells by the growth of the barbs and barbules (Strong, 1902; Hosker, 1936). One barb, dorsally located, is somewhat larger than the rest, having been formed by fusion of two or more ridges (Hosker, 1936); this is the rachis (cf. Fig. 377-G; Fig. 378-A<sub>2</sub>). It is more pronounced in the



down of the duckling than in that of the chick. Hosker (1936) also identified an aftershaft in the down as a short ventral rachis with a few barbs attached to it.

Barbules attach to barbs at their ends by means of a barbule cell which is elongated radially rather than axially. At 18 days in the embryo duck (*Anas platyrhynchos*), the feather sheath, composed of the epitrichial layer, is partly cornified, with only occasional nuclei in evidence (cf. Fig. 377-F). Breaks in the sheath were observed by Hosker (1936) 3 days prior to this time (cf. Fig. 377-C). At 19 days, the cylinder cell layer has begun to withdraw from between the barb ridges. An additional barb joins the rachis, forming a "branch." On the twentieth day, the cells of the rachis have developed large vacuoles; the smaller barbs follow. Between the twenty-second and twenty-fourth days of the duck (*Anas platyrhynchos*) the large barbs on the upper side of the feather fuse together with the rachis (cf. Fig. 377-G), and the hyporachis originates by unification of ventral barbs. Proximal fusion of the barbs becomes extensive on the following day, and by means of fusion of the shaft and aftershaft, the calamus or quill is formed by the twenty-fourth day of incubation. The follicle has deepened by down-growth of the epidermis; the epitrichial layers of follicle and feather have become continuous by proliferation in the basal region, near the quill. Keratinization is beginning here, and is already complete in the feather tip (Hosker, 1936). The process is initiated by a radial coalescence of cells; their walls become thickened, and nuclei are finally compressed into a thin line.

From the fifteenth day until hatching (cf. Fig. 377-C, D, and F), the pulp is gradually withdrawn to the base of the feather (Hosker, 1936). During this process cylinder cells may be stranded in the feather shaft, and may then become cornified as "feather caps."

**The definitive feather.** Feathers having the adult morphology are present on the posterior margin of the wing of the newly hatched chick. These are a row of flight feathers and their major coverts; they bear the replaced down feathers on their tips, since some of the barbules are continuous through both structures (cf. Fig. 378-B<sub>1</sub>). These are the first representatives of the juvenile plumage, which appears gradually in a definite sequence in all the pterylae during the first 5 weeks of the chick's life (Chu, 1938). In the duckling (*Anas platyrhynchos*), juvenile feathers appear first in the tail at about 2 weeks, and wing flight feathers come in at about 5 weeks. Chick plumage is replaced gradually in the same order in which the juvenile plumage emerges, and in the latter coat sexual dimorphism is first expressed in the feather patterns (Chu, 1938).

Development of the definitive feather is essentially similar to that of the down. A primordium of a penna has many more ridges than does a down primordium, although toward the end of the formative process the barbs



and barbules may differentiate before ridges have been formed (*Hosker, 1936*). The sheath or stratum corneum has up to 10 layers (*Hosker, 1936*).

Primary ridges are formed in such a manner that the barbs grow more dorsally than proximally, soon meeting and fusing with the rachis. The distal ends of new barbs arise in the intermediate cell layer at the ventral point of the feather, and are again oriented toward the rachis so that addition of cells from the proximal region causes them to elongate in the direction of the shaft. In the proximal portion of the feather, the region of origin of barbs is displaced dorsally about one third of the way along the circumference of the papilla, and from this locus, known as the region of plasmatic growth, barbs may grow ventrally toward the hyporachis.

Lillie and Juhn (1932, 1938) have hypothesized that the rachis exerts an attractive influence on the growing barbs, and that the aftershaft results only after a weakening of the attraction (the "rachis gradient"). Lillie and Wang (1944) have shown that the dorsal portion of a regenerating dermal papilla can, when transplanted to the ventral region of another papilla, cause a mirror-image duplicate of the normally occurring feather to be built. The capacity to do this decreases in grafts taken more ventrally along the circumference of the germ, vanishing in tissue taken from the equator.

**Movement of the feather muscles.** Each definitive feather follicle usually has four smooth, small, unstriated muscles arising within the dermis. Sometimes there may be one or two more. They are situated anterolaterally, posterolaterally, and longitudinally from the middorsal line passing almost exclusively between the roots of the feathers, being attached to them by tendons at each end. Their purpose is to erect, depress, or retract the feathers (*Langley, 1904*).

### *The Beak Region*

The beak is first barely recognizable in a chick embryo at 5.5 days (*Kingsbury, Allen, and Rotheram, 1953*) as a thickened ectoderm of the middorsal boundary of the oral cavity where the nasomedial processes have fused and grown forward. By the next day the epiderm of the mandibles is more developed than elsewhere, the embryonic head being somewhat transparent except at the anterior end of the upper jaw (*Gardiner, 1885*) where a small distinct opaque outgrowth is apparent, this being the prospective egg tooth.

**Structural Development of the Beak.** The beak becomes more pronounced and shows the egg tooth on the seventh day. Cornification of the beak begins on the tenth day and by the fourteenth day the upper beak begins to have a hard surface, starting near the egg tooth region and spreading to the tip. In the lower jaw cornification also takes place beginning near the tip of the beak.



The cornification of the beak involves many cell layers and takes place by rapid proliferation of the cells in the mucous layer of the epidermis. The cells are polyhedral in the intermediate layers and flattened superficially. In the embryonic beak keratin develops in the stratum granulosum (Lewin, 1903) of the outermost epidermal layer, the epitrichium or periderm, which is shed before hatching. By the ninth day the periderm consists of from five to seven layers of cells. As the beak increases in size the peridermal covering increases. The individual cells enlarge and their numbers are increased by additions from the stratum germinativum. At first these newly formed peridermal cells are added along the extent of the germinativum, but later only at the anterior, lateral, and posterior margins of the cornifying beak, where the periderm does not form a distinct layer but is contiguous with the rapidly proliferating cells of the germinativum. The portion of the beak nearest the egg tooth contains the oldest peridermal cells (Kingsbury, Allen, and Rotheram, 1953), as well as the oldest and thickest cornified layers.

Carotinoid pigments occur in the epidermal cells of the beak at the onset of keratinization in the form of lipochromes and account for the yellow color of the beak.

In the last 2 embryonic days of the chick the translucent peridermal covering of the beak starts peeling, the beak shortens through this sloughing off of its entire periderm (Hamburger and Hamilton, 1951), and is blunt at its tip and shiny at hatching. The growth of the beak from the anterior angle of the nostril to the tip of the beak is shown in the tabulation.

Age of Embryo (days)	Length of Beak (in millimeters)	
	Heavy Breed (Byczkowska, 1953)	Light Breed (Hamburger and Hamilton, 1951)
10	3.3	2.5
11	3.7	3.0
12	4.0	3.1
13	5.0	3.5
14	6.0	4.0
15	7.0	4.5
16	8.0	4.8
17	9.0	5.0
18	10.0	5.7
19	11.0	5.7
20	11.0	5.7—
21	11.0	

The shortening of the beak after the loss of the periderm is accounted for by the fact that as the beak is formed, the stratum corneum, which formed first in the region of the egg tooth, extended between the periderm and



underlying germinativum in both jaws (Kingsbury, Allen, and Rotheram, 1953) but not to the distal end of the jaws.

The periderm of the beak may form a protective covering from the amniotic fluid since water causes a swelling of cornified cells. As the amount of fluid decreases, there is a correlation between a thickening of the stratum corneum and the progressive degenerative changes in the periderm, so that when the periderm is sloughed off about 2 days prior to hatching (Kingsbury, Allen, and Rotheram, 1953), no amniotic fluid remains.

**The egg tooth.** The egg tooth is a transitory, horny structure with pointed nipple on the dorsal surface near the tip of the upper beak of birds. It is used by the chick on hatching to break the shell membrane. The egg tooth is lost by snipes (*Gallinago gallinago*) and woodcocks (*Scolopax rusticola*) and by ducks (*Anas platyrhynchos*), geese (*Anser anser*), swans (*Cygnus* sp.), and mergansers (*Merginae*) a few hours after hatching. In bustards (*Otis*) and penguins (*Spheniscidae*) it persists for a few weeks (Stresemann, 1934; Rezovska, 1934).

It is first visible as a conspicuous, opaque, rounded protuberance in the upper jaw about the seventh day of incubation (Gardiner, 1885; Byczkowska, 1953; Kingsbury, Allen, and Rotheram, 1953). It develops from the basal epithelium, becoming horny from the rapidly growing stratum germinativum (Rezovska, 1934). The ectoderm of this section consists of seven or eight layers of polygonal cells with large nuclei and distinct outlines and is the primordium of the egg tooth.

In some species of birds, as in snipes (*Gallinago gallinago*) and lapwings (*Vanellus vanellus*), there is present microscopically a small, light gray or white egg tooth at the anterior end of the lower bill (Rezovska, 1934). Both upper and lower egg tooth are the same in structure but the latter is broader at the base, develops embryonically on the thirteenth day (Byczkowska, 1953), and does not attain the same size (Willink, 1899). The egg teeth are not comparable with regular teeth in origin or development (Rezovska, 1934).

The egg tooth develops like the beak (Lewin, 1903), except that the cells become horny and yellow earlier than those of the beak. After its first appearance the egg tooth increases rapidly and protrudes over the dorsal surface of the beak by the tenth incubation day. The cell layer which covers the tooth early in development ruptures on the eleventh day of incubation. The beak grows more rapidly than the egg tooth making the tooth, at early incubation stages, appear much bigger.

At first the epitrichium in the egg tooth region contains no keratin nuclei. In a few days the epitrichium on the distal part of the tooth attains half the thickness of the tooth. The largest keratin nuclei develop in the epitrichium of the tip (Rezovska, 1934) and these decrease in number and size distally. As the egg tooth grows, the epitrichium begins to shrink,



especially on the tip where it is very thin by the fifteenth day (*Rezovska, 1934*). Degenerating nuclei are present in cells nearest the stratum corneum as horny cells continue to form. The egg tooth attains its maximum size between the fourteenth and fifteenth day of incubation (*Byczkowska, 1953*) or, according to *Kingsbury, Allen, and Rotheram (1953)*, at about the sixteenth day. The outer covering of the egg tooth, called the periderm, is a horny structure which progressively degenerates and is sloughed off about the nineteenth day of incubation (*Kingsbury, Allen, and Rotheram, 1953*), leaving a thick stratum corneum. The accompanying table shows the length of the egg tooth in millimeters in a heavy breed chick (*Byczkowska, 1953*).

Age of Embryo (days)	Egg Tooth Length (mm.)
10	3.0
11	3.3
12	3.4
13	3.4
14	3.5
15	3.5
16	3.5
17	3.5
18	3.5
19	3.5
20	3.5
21	3.5

Under the base of the egg tooth a dense network of capillary vessels are formed enabling the rapid growth of the basal epithelial cells which constitute the egg tooth (*Byczkowska, 1953*) but none penetrate the egg tooth itself. The capillary vessels situated below the epithelium of the other parts of the beak are narrower than those at the base of the tooth.

Some irregular grooves and protuberances appear on the upper layer of the tissue of the egg tooth of some species of Aves but not in snipes (*Gallinago gallinago*) and chickens. These grooves are more pronounced in earlier embryonic stages, and later are visible only on the sides and base of the egg tooth. The significance of this irregular surface is not known (*Rezovska, 1934*).

**The labial groove.** A deep furrow at the tip of each jaw appears at 12 days (*Gardiner, 1885; Hamburger and Hamilton, 1951*) above the upper edge of the beak and parallel with it. This labial groove is an invagination of the epidermis observed in chick embryos and in the zebra parakeet (*Melopsittacus undulatus*) but not in the duck (*Anas platyrhynchos*). Phylogenetically, the labial grooves have been compared with the lips of other vertebrates. In the kite, *Milvus ictinus*, and hawk, *Accipiter nisus* (*Gardiner, 1885*), the upper labial groove occurs within the mouth cavity and in the dove, *Columba livia*, it is an invagination on the tip of the beak.



As the groove deepens, the horny process continues and the labial edges grow closer together. The groove in the lower jaw is similar but much smaller and more shallow than in the upper jaw (*Gardiner, 1885*). By the seventeenth day of incubation these grooves are reduced to a white granular crust, that of the lower jaw already having been or beginning to be sloughed off (*Hamburger and Hamilton, 1951*). The labial groove demarcation remains after hatching (*Gardiner, 1885*) in the case of the chick and in the zebra parakeet (*Melopsittacus undulatus*).

**The beak cushion.** In nestlings such as English sparrows (*Passer d. domesticus*) and hoopoes (*Upupa*) the beak cushion is a small fold of skin connecting the upper and lower beak and extending to the nostrils, in some cases. It serves as a landmark in the feeding of juvenile nestlings by

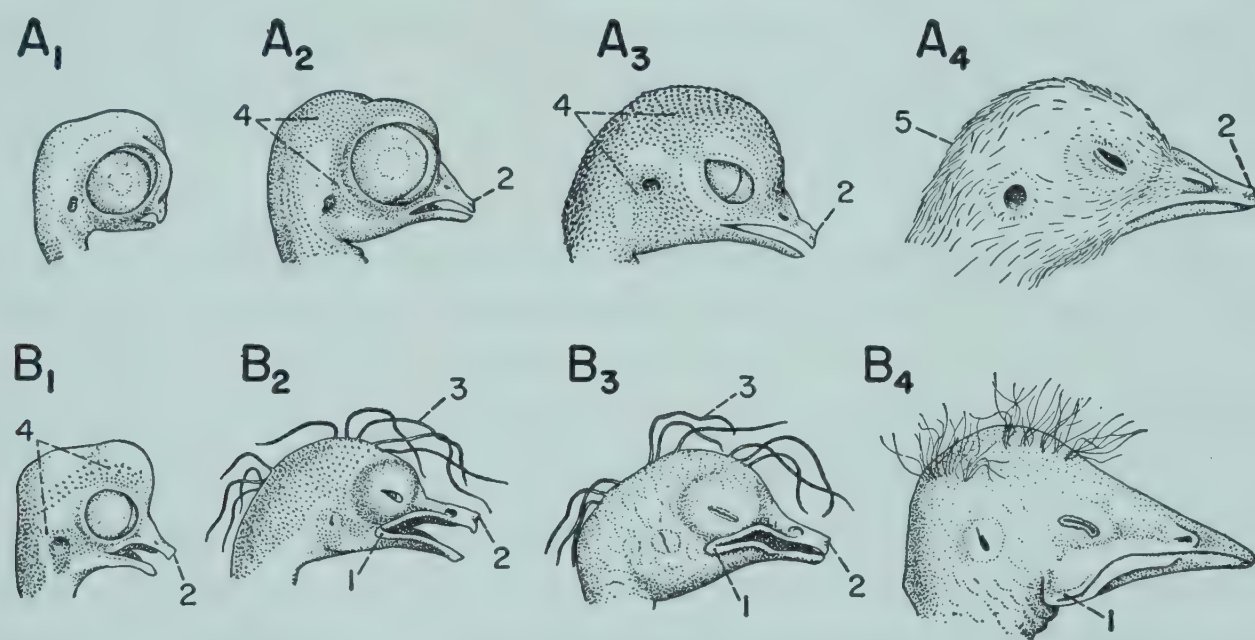


Fig. 379. Comparison between a chick (*Gallus gallus*), a typical precocial bird, and a starling (*Sturnus vulgaris*), an altricial bird, showing beak development and in the latter, the beak cushion. (Redrawn with modifications after Portmann, 1939.)

A<sub>1</sub>, chick at 7 days' incubation; A<sub>2</sub>, at 10 days; A<sub>3</sub>, at 12 days; A<sub>4</sub>, at 18 days; B<sub>1</sub>, starling at 7 days' incubation; B<sub>2</sub>, at 10 days; B<sub>3</sub>, at 12 days; B<sub>4</sub>, as a nestling.

1, beak cushion; 2, egg tooth; 3, prenatal plumage; 4, feather tracts; 5, down.

the adult bird (*Stresemann, 1934; Wackernagel, 1954*). In general, it is conspicuously yellow or white, but in the hedge sparrow (*Prunella modularis*) is light pink. The structure atrophies (*Portmann, 1950*) after the nestling becomes independent. Although embryonic development of the beak cushion of various nestlings is the same, juvenile development differs.

The beak cushion is derived from the relatively smooth, moist mucous layer. According to Wackernagel (1954), it is first evident in the starling (*Sturnus vulgaris*) embryos (incubation period 13 days) and in lapwings (*Vanellus vanellus*) on the seventh day (Fig. 379-B<sub>1</sub>) and in crows (*Corvus cornix*) on the eleventh day as a small, protruding fold in the corner of the mouth. On the seventh day of the starling (*Sturnus vulgaris*) embryo the cushion is filled with regular, typical embryonic mesenchyme (*Wacker-*



nagel, 1954). The cell structure is similar to that of the skin of the head. The ectoderm consists of two layers of protoplasmic cells with large oval nuclei, which contain one to three large nucleoli, and a layer of flat cells with small, long nuclei. A basal membrane borders on the ectoderm.

Two days later the first, fine collagen fibers arise in the cushions becoming more pronounced the next day than in the surrounding area. These fibers intertwine in the mesenchyme and underlie the ectoderm, coming close to the basal membrane. The nuclei of these collagen fibers become enlarged as do the nucleoli.

The ectoderm on the tenth day is still as it was on the seventh day but is becoming thicker distally in layers and is especially strong at the surface of the cushion where there are four to five layers of nuclei. The basal nuclei are elongated, the center ones are round, and above these are elongated nuclei lying parallel with the surface. In the upper and middle layers of nuclei, cell boundaries are becoming apparent, followed a little later by the same process in the basal nuclei. Also, the cells in the top layer have flattened and separated.

The beak cushion grows rapidly and becomes higher and thicker so that by hatching time (cf. Fig. 379-B<sub>3</sub>) it occupies two thirds of the beak rim. The collagen fibers have become long strands closely woven among the fibroblasts whose nuclei appear as on the tenth day. The upper two to three layers of cells laden with keratin and known as the periderm are shed, leaving a typical horny epidermis of about eight layers compared with the approximate five layers of the feathered head area. By hatching the chief characteristics of the beak cushion are recognizable. The swollen folds are widest at the mouth corner where the upper and lower folds blend together, the under beak fold being broader and longer (Fig. 379-B<sub>4</sub>). A cadmium yellow appears for the first time on the last day of incubation of the starling (*Sturnus vulgaris*) in the mouth areas and becomes stronger after hatching.

### *Scales, Spurs, and Claws*

**Leg scales and foot pads.** These are of greatly thickened stratum corneum resulting from the highly keratinized cells of the germinative layer of the epidermis. Scales are first formed by a thickening of dermal cells and are therefore of mesodermal origin. In the chick, the first sign of scales appears between the tenth and eleventh days of incubation (Jeffries, 1884; Hamburger and Hamilton, 1951) on the superior surfaces of the metatarsus and phalanges. The primordia of scales are evident over the entire leg surface by the next day (Hamburger and Hamilton, 1951).

The skin on the tarsus and toes before the actual appearance of scales consists of an epitrichial layer of flattened polygonal cells. Under this are the closely packed protoplasmic mucous cells, which are cuboidal to col-



ummar in form and have thick cell walls (*Kerbert, 1877*). These lie next to the dermis. Between these two layers is a layer of polyhedral and closely packed cells. By the end of the twelfth day of incubation (*Jeffries, 1884*) the ridges fold toward the tip of the limb, growing in a bilateral symmetrical manner. The cells next to the epitrichial layer are now fusiform, being more numerous on the tarsus than on the toes and are still protoplasmic without structural characteristics of horn. The mucous layer is still much the same, the cells varying from columnar to cuboidal depending on their progress in division. The epitrichial cells are spread out. By the fifteenth day the fold of the scale has lengthened. The epitrichial layer is now distinctly a layer of cells.

On the nineteenth day the epitrichial layer has assumed its definitive form of irregular polygonal cells, the nuclei are indistinct and the contents granular. Below this layer the granular layer remains the same showing a tendency to split off from the horn cells below, which have become more numerous.

From now until hatching no important changes occur in the epidermis. The horn cells continue to proliferate from the mucous layer and mature. At hatching the epitrichial and granular layers are shed (*Jeffries, 1884*) and the scales are in their final condition.

Some pigment cells, the same as those in feathers, are to be seen in the dermis but these disappear by the nineteenth day (*Kerbert, 1877*). The dermal component remains vascular.

Pads on the plantar surface of toes are comparable to scales but do not overlap. They remain as polygonal papillae to give nonskid surface to the sole of the foot. In the chick embryo they are conspicuous and smooth at 11 days. The next day the plantar pad is ridged. At 13 days the major pads of phalanges are covered with papillae and the minor pads are smooth. At 14 days (*Hamburger and Hamilton, 1951*) the entire plantar surface of phalanges is covered with well-developed papillae.

**Spurs on legs and wings.** The spurs are conical formations on the inner or on the dorsal part of the legs and are composed of a bony core covered by the cutis vera and epiderm, the bone being attached to the subjacent tarsometatarsal bone by ossification after hatching.

They are of mesodermal origin being formed like scales by a thickening of dermal cells after which great proliferation in the germinative layer of the epidermis takes place and a consequent increase in the stratum corneum. The first appearance of the spur in the chick is on the tenth day of incubation as a circular swelling on each leg proximal to the first toe. The spur is of the same structure as the surrounding area of scales but does not form a fold.

Some species of *Aves* have spurs on the wing in the vicinity of the carpal joint. They are found in the blackbird (*Turdus merula*), many of the geese



(*Anser anser*), pigeons (*Columba livia*), jacana (*Parra* sp.), jungle fowls (*Gallus gallus*), and in the horned screamer (*Anhima cornuta*), which has two spurs (Jeffries, 1883).

**Claws of legs and wings.** Claws of toes are visible at 10 days (Hamburger and Hamilton, 1951). At 11 days they are flattened laterally and curved ventrally; the dorsal tips are opaque (Hamburger and Hamilton, 1951) indicating onset of cornification.

On the anterior edge of the wing of birds there are thumblike projections or claws. They are smooth and inconspicuous having no special function in adult birds and are therefore aborted organs. The tapering primordia of claws are visible on the terminus of digit one of the wing of the chick at 10 days' incubation (Hamburger and Hamilton, 1951) and on the next day are opaque.

### Vascularized Structures

Vascularized structures are found where the epidermis is very thin and the dermis is supplied with a dense vascular and lymphatic tissue, showing the color of blood through the surface. The comb and wattles are of this structure and are very sensitive, reflecting the physiological condition of the bird.

**The Comb.** The comb of birds extending longitudinally from the beak posteriorly and with the nostril slits as the ventral boundary shows its highly vascular dermal structure through the thin epidermis. Of the several easily identified comb types of domestic fowl only the single comb will be considered here. Throughout embryonic life male and female combs are indistinguishable (Hardesty, 1931).

The primordium of the comb in the chick first appears as a localized thickening of the comb region caused by the rapidly proliferating mesenchyme cells of the mesoderm between the sixth and seventh days of incubation (Stephenson, 1915; Hardesty, 1931).

A few hours after the first appearance of comb elevation small papillae are evident along the middle and posterior parts of the comb region. These papillae are the primordia of the serrated adult comb, one of the chief characteristics of most single combs. There are no feather germs in this area and in a few days it is clearly differentiated from the beak. The line of demarcation is notched (Hardesty, 1931) and remains so throughout the life of the bird.

The comb ridge continues to develop in width and height (Stephenson, 1915) accompanied by a thinning of the ectoderm and rapid growth of underlying mesodermal cells with an influx of blood vessels. Up until 12 days of incubation the median comb elevation extends anteriorly to the beak occupying half of the comb area longitudinally and one third of the transverse area.



**Structural development.** At first signs of comb elevation the epidermis consists of two layers, the epitrichial layer and the mucous layer (Kerbert, 1877). From this mucous layer develops the definitive epidermal mucous layer and the transitional layer, which, along with the epitrichial layer, contribute to the formation of the horny layer. For some time the area around the comb continues in the early stages of transitional layer cell formation while within the comb area active cell division results in four or five layers of cells by the thirteenth day and gives rise to the transitional layer of the definitive comb epidermis.

Meanwhile, the mucous layer cells have changed from round to cylindrical shape and the horny layer has started from the proliferating and flattening transitional layer cells. By the eighteenth day the epidermis has assumed adult appearance with horny cells at the periphery.

The dermis at 7 days shows the mesenchyme of the comb ridge more compact and composed of round cells (Hardesty, 1931). These become elongated and spindle-shaped the next day which is the beginning of the central connective tissue characteristic of the adult comb (Stephenson, 1915). Stratification is evident by the tenth day. As the comb heightens in succeeding days the fibers become vertical and parallel with each other in the core as rapid proliferation of mesenchyme cells continues. Its two lateral areas move mesiad and the comb becomes relatively thinner in proportion to its height. The small blood vessels previously scattered through the basal region of comb elevation move into the comb itself spreading branchlike. The embryo is in this condition at hatching (Stephenson, 1915; Hardesty, 1931).

**Effect of testosterone preparations.** The comb of a chick at hatching, the egg having been injected with testosterone-propionate on the fourth day of incubation, is thick at its base, elevated, and turgescient, but still pale (Danchakoff and Kinderis, 1937).

**Wattles.** Wattles, like the comb, are epidermal folds of mesodermal origin. Their first appearance (Jeffries, 1884) is at 11 days of incubation. The epidermis, which is thin, is composed of mucous, transitional, and flat horn cells.

### Pigmentation

Coloration of the integuments in avian species is provided by two classes of pigments, the melanins and the lipochromes. The first class is produced by specific chromophores which transfer their pigment granules to the feathers (Fig. 380); the second is associated with the Malpighian layer of the epidermis, and is most important in featherless areas, although it may also color the feathers in such birds as the canary (*Serinus canaria*). Melanophores occur also in the gonads of birds, usually in asymmetrical distribution favoring the left side. In the Silky fowl (*Gallus gallus*) mela-



nophores are absent in the feathers, but occur in the peritoneal layers, in the stroma of many of the viscera, in the meninges, periosteal, dermis, and epidermis (Kuklenski, 1915).

**Derivation of melanoblasts.** Controversy existed for many years as to the origin of the chromatophores. It was maintained by some workers (Rabl, 1894; Strong, 1902; Hosker, 1936) that they are modified epidermal cells; others claimed that they migrated into the epidermis from the dermal layer. Origin of vertebrate pigment cells from the neural crest was proposed in 1912 by Weidenreich, and has recently been proved experimentally to be the case in birds. Explants of neural crest from chickens of various breeds produce pigment when cultivated *in vitro* (Dorris, 1938b). Grafts of neural crest material from embryos which will have black or red feathers to the

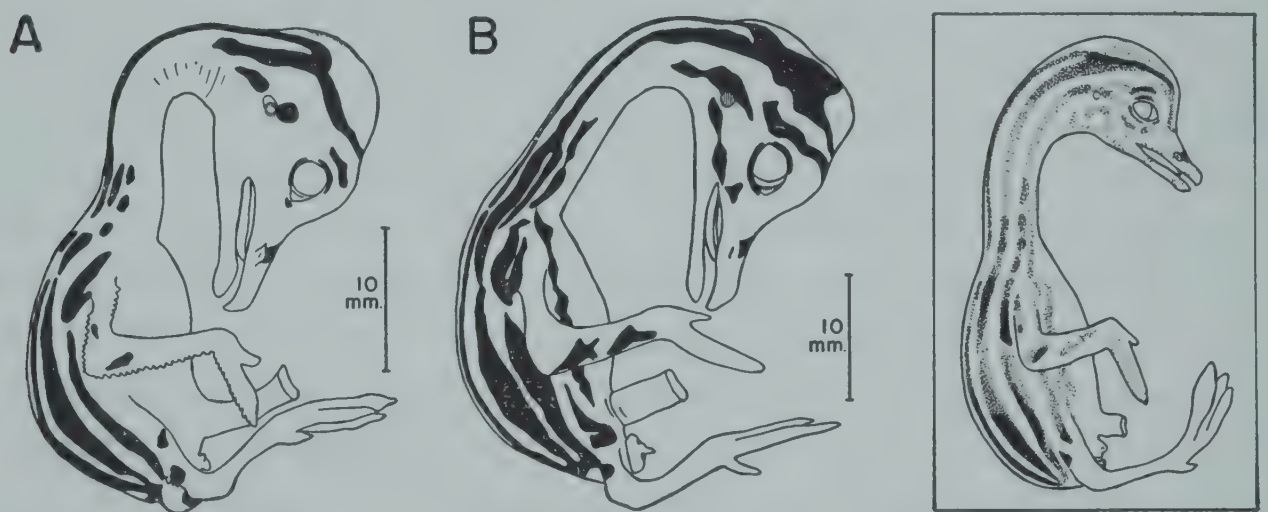


Fig. 380. Development of feather pigmentation tracts in the crested grebe (*Podiceps cristatus*) embryo. (Redrawn with modifications after Portmann and Gerber, 1935.)

A, at 16 days' incubation; B, at 17 days. Both in scale.

*Insert:* diagram of pigmentation tracts, indicating the relative times of appearance of pigment; the first areas to appear are shown in black, with later developing zones shown progressively lighter.

limb area of white-feathered breeds cause the formation of a donor-colored patch of feathers on the limb (Dorris, 1939). Early ectodermal isolates produce pigment only if neural crest is included (Ris, 1941). Tissue grafted from the wing and leg areas alone is first able to produce a donor-colored patch in the limb of a host embryo at 80 and 96 hours, respectively (Willier and Rawles, 1940). A similar ability to transfer the donor color to a white host embryo is acquired in a definite time sequence by various parts of the epidermis, beginning anteriorly and in proximity to the neural tube and progressing laterally and toward the caudal end of the body; the lateral extension of this capacity appears first in the rapidly growing limb areas (Fox, 1949).

In the phase of their differentiation the potential pigment cells can be identified only with difficulty. A cell type which has a similar distribution



to the melanoblast from 20 hours to 12 days of incubation has a strong affinity for Nile blue sulfate and neutral red, but isolates of these cells have a limited capacity to synthesize melanin and often assume a form characteristic of tissue mast cells (*Saunders, 1949*). Watterson (1942) and White and Eastlick (1953) have described an amoeboid cell, having little cytoplasm, an elongated or rhomboid nucleus, and a single enlarged, eccentrically located nucleolus, which was observed at about 80 hours in the dermis of the wing bud. In the Barred Rock fowl (*Gallus gallus*), some of these cells have cytoplasmic processes extending into the epidermal layer at this time (Watterson, 1942), and a similar migration occurs in the Silky fowl (*Gallus gallus*) at about 90 hours (White and Eastlick, 1953). The number of migrating cells appears to be small; there were from two to five in each pair of wing buds fixed at this time, increasing to a maximum of seven to eight at 82 hours (Watterson, 1942). At 90 hours cells of similar morphology are found in the epidermis of the wing, their long axes parallel to its surface (Watterson, 1942); and there is no longer evidence of amoeboid movement in the form of an irregular outline (White and Eastlick, 1953). At this stage, pigment can be obtained from isolated wing buds (Willier and Rawles, 1940).

**Differentiation of melanophores.** Synthesis of melanin by definitive melanophores begins between 7 and 7.5 days in the Barred Rock embryo, *Gallus gallus* (Watterson, 1942), and at 8 days in the Silky fowl, *Gallus gallus* (White and Eastlick, 1953). The number of cells synthesizing pigment indicates that the melanoblasts have proliferated extensively in the 3 days since their establishment in the epidermis. Mitosis may also occur in definitive melanophores which have begun to synthesize pigment (Watterson, 1942). The nuclei of these cells round up, and one or more cytoplasmic processes are projected (White and Eastlick, 1953). Melanin is laid down in the form of granules having a shape characteristic of the species and breed of the bird (Willier and Rawles, 1938); these granules are at first very thin and highly refractile, but they darken markedly as material is added to them (White and Eastlick, 1953). Differentiation of the melanophores has been observed by Strong (1902) in the regenerating adult feather.

**Genetic effects on melanophore differentiation.** Melanophores, grafted either from the epidermis or neural crest of pigmented embryos into white hosts, generally reproduce not only the color but also the pattern typical of the donor breed (Rawles, 1939; Danforth, 1939; Willier and Rawles, 1940). It has been suggested by Nickerson (1944) that barring found in definitive feathers of both the Barred Plymouth Rock (*Gallus gallus*) and the Silver Campine fowl (*Gallus gallus*) is dependent upon a diffusible inhibitor of melanophore differentiation. Histological examination of a white region of the regenerating feather germ reveals that melanophores



are not present in the epidermal tissue which will produce barbs and barbules; but if this presumptive white band is transplanted to the limb bud of a White Leghorn (*Gallus gallus*), it can produce a barred pattern. The phase of the barring pattern formed at any time is correlated with the length of time since the beginning of pigment deposition rather than the total age of the feather, indicating that definitive melanophores are probably the producers of the inhibitor.

In the Barred Plymouth Rock (*Gallus gallus*), the barring pattern also exhibits sexual dimorphism. The barring gene is a sex-linked dominant, and as homozygous males have two barring genes they should have less melanin than homozygous females (*Spillman, 1909*). The difference is very obvious in the feather papillae of the spinal tract on the tenth day of incubation (*Watterson, 1942*); males and females are less markedly different on the following days of development.

In both dominant and recessive white breeds of fowl, melanophores are present at early stages in formation of feather papillae, but when barbules begin to differentiate the cells round up and die (*Willier and Rawles, 1940*). The cells evidently are a lethal strain, as they do not survive any better when transplanted to pigmented hosts.

**Deposition of pigment.** At the end of the tenth day of incubation of the Barred Rock embryo, *Gallus gallus* (*Watterson, 1942*), the melanophores in the feather germs are packed with pigment granules. Late on the tenth day, granules begin to accumulate at the tips of the cell processes, and blebs of pigment pinched off from these cells may be found among the intermediate cells of the developing feather primordia. On the eleventh day of incubation, they take up a position along the inner margins of the ridges which will rise to feather barbules. A cross section of the feather germ at this time shows that these large cells have processes leading to the most peripheral barbule cells, which are the first to differentiate; there seems to be a specific attraction in operation (*Watterson, 1942*). As barbule cells complete their keratinization, the melanophore processes withdraw to more axially located barbules (*Strong, 1902; Watterson, 1942*).

Apparently, those cells which have furnished pigment to the barbules deposit it also in the barbs. When the barb becomes keratinized, melanophores are trapped in its distal third. In the proximal two thirds of the germ the pigment cells withdraw to the dermal layer, round up, and retract along with the pulp (*Watterson, 1942*).

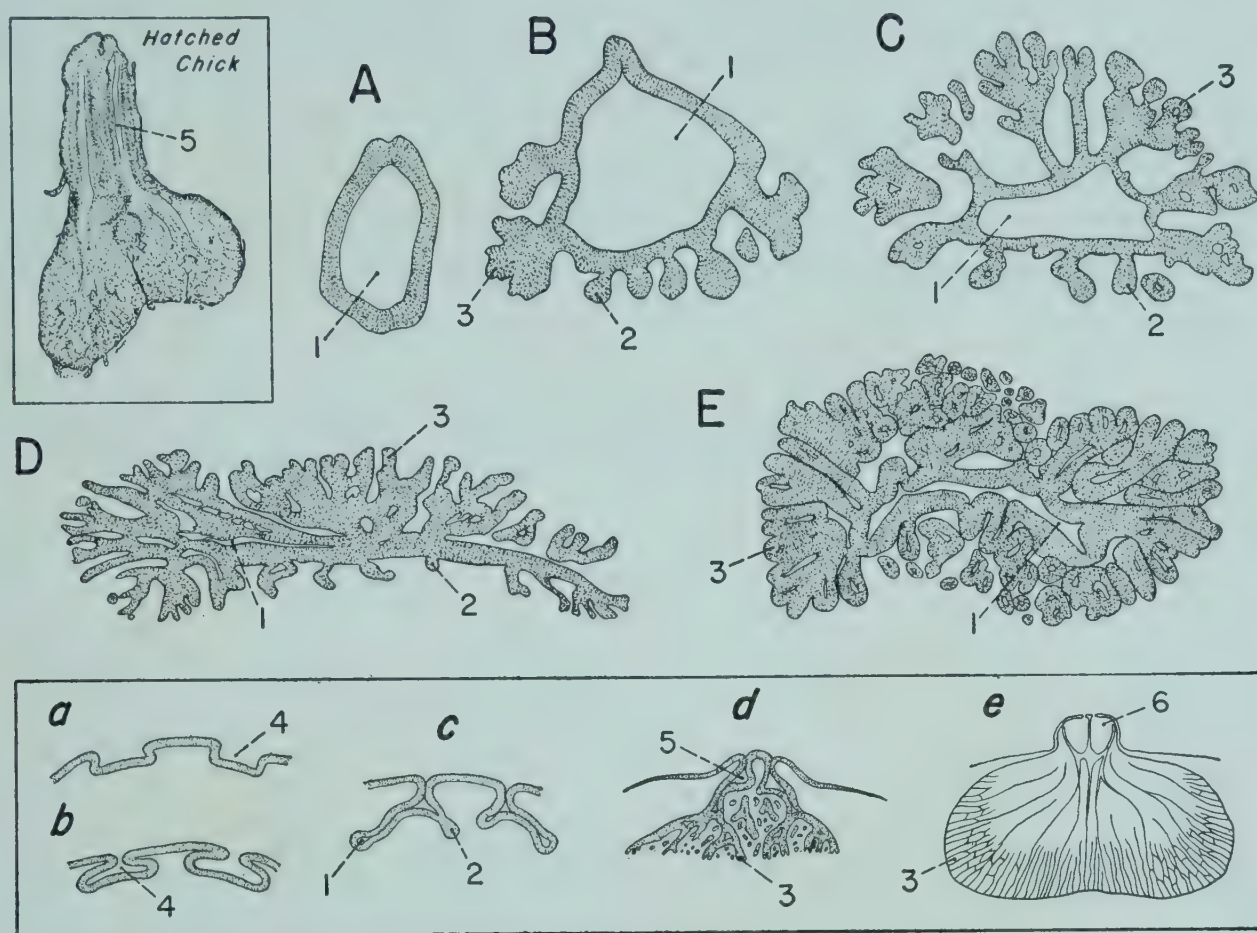
### The Uropygial Gland

The uropygial gland is the only important integumentary gland of birds. It is vestigial or absent in some birds of prey (*Cater and Lawrie, 1950*) and is situated on either side of the coccygeal vertebrae at the root of the tail feathers. It is a bilobed, spongy structure terminating in a teat with two



openings. Each lobe contains a small central cavity with numerous, radially arranged tubules which discharge an oily secretion. It is used by the bird by means of its beak to preen and waterproof its feathers. This gland is not comparable with the common glands and it first functions several weeks after hatching.

**Origin.** It is of ectodermal origin and its first appearance in the chick embryo is evident at 9.5 days as a modification of the rump region epidermis consisting of an upper layer of flat, pressed cells with clearly visible nuclei



**Fig. 381.** Semidiagrammatic representation of stages in the development of the uropygial gland in the chick embryo. (Redrawn with modifications after Lunghetti, 1907; Cater and Lawrie, 1950.)

*Insert, at top:* section through the uropygial gland of a day-old chick.

A, cross section through the uropygial gland of an 11-day embryo; B, at 13 days; C, at 15 days; D, at 19 days; E, at 21 days.

*Insert, a-e:* diagrammatic representation of stages in the development of the uropygial gland in the sparrow (*Passer domesticus*).

1, lumen; 2, primary buds of gland; 3, secondary buds; 4, first invaginations of gland; 5, duct; 6, teat.

and a lower layer of cylindrical cells. An early change occurs in the lower layer, the cells of which become higher and thicker (Lunghetti, 1907). The region, level up to now, sinks in, and by the tenth day (Fig. 381) there are two long indentations or double invaginations very close together posteriorly and somewhat separated anteriorly (Kossmann, 1871; Lunghetti, 1907; Cohn, 1952).

**Development.** The smooth walls of the forming pits have an epithelium typical of the skin. The floor has a layer of cylindrical cells noticeably



thicker than that of the walls. In the next few days the rims of the groove converge, covering both cavities. The buds of the teat feather arise in the rim (*Kossmann, 1871; Lunghetti, 1907*).

Important changes in the cavity epithelium take place. The cylindrical cells in various places of the wall proliferate actively and build firm buds of polygonal epithelial cells. These are compact, solid ridges which shove into the surrounding embryonic tissue and soon hollow out. They are the source of the sponge section. Many close branches arise from these primary buds. They are thin, long epithelial cones, called secondary buds, are firm at first, and have many vascular germs. The tubules increase in number and begin to develop lumina up until hatching (*Lunghetti, 1907*). They show lipids in their surface cells which continue to accumulate in the deeper lying cells from the sixteenth day of incubation to hatching, along with vacuolation and disintegration of these cells which produce the secretion (*Cohn, 1952*).



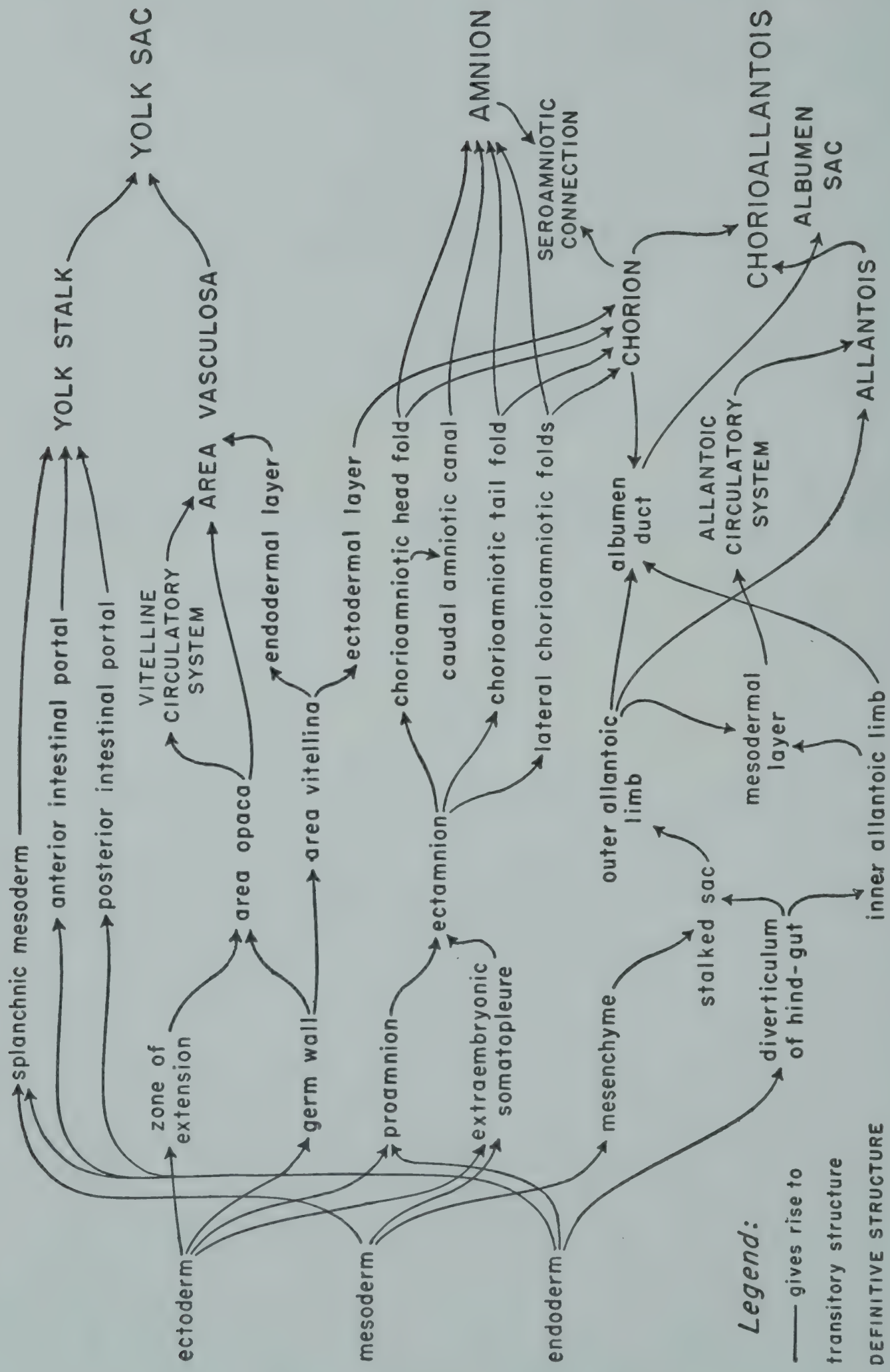
## CHAPTER THIRTEEN

# *The Extraembryonic Membranes*

*Their role is wholly embryonic.*

*Each is a functional machine,  
Protects from harm, gives vital tonic.  
Such action nowhere else is seen.*







# THE EXTRAEMBRYONIC MEMBRANES

In the higher vertebrates, including birds, the development of the embryo cannot occur without the mediation of temporary appendages that persist until the transition to independent existence becomes possible. These appendages are the extraembryonic membranes. In birds, they consist of the yolk sac, the amnion, the chorion, and the allantois; together, the latter two form the chorioallantois, an extension of which encloses the albumen and is known as the albumen sac. Although they are living structures, derived from the blastoderm and continuous with the tissues of the embryonic body, the membranes are semiautonomous; and each is endowed with distinctive morphological and physiological attributes in accordance with its role in development. The functions of the membranes are the vital ones of nourishment, protection, respiration, and the segregation of waste products. It is through the membranes that the embryo is placed in a favorable relationship with its total environment, which includes the environment within the egg as well as the world beyond the limits of the eggshell.

All of the blastoderm which is not incorporated into the embryo proper is utilized in the formation of the extraembryonic membranes. In the beginning, the relationship of the membranes to each other and to the embryo is fairly simple. As development proceeds, the membranes enlarge and spread throughout all the space that is available to them, becoming complexly interrelated, and, in some areas, intimately fused together. Eventually, they form a system of considerable complexity. At the end of incubation, they all undergo involution with the exception of the yolk sac, which persists for a variable time after hatching.

## THE ORIGIN OF THE EXTRAEMBRYONIC MEMBRANES

The boundary between the embryonic and extraembryonic portions of the blastoderm cannot be fixed with certainty until a relatively advanced stage of development, when the body has been completely folded off. Long before this time, however, it is possible to observe the initial processes in the formation of the extraembryonic membranes. Furthermore, the area opaca (the region of the germ wall, the zone of junction, and the margin of overgrowth) can be considered at any time as extraembryonic.

Essential processes in the development of the membranes are the insertion of mesoblast between epiblast and hypoblast and the horizontal cleavage of the mesoblast into an upper, or somatic, and a lower, or splanchnic, layer. At the stage of the definitive primitive streak, the meso-



blast, spreading out from the streak into the area pellucida, begins to encroach upon the area opaca along the posterior and posterolateral borders of the area pellucida. During head-process stages, this encroachment reaches the level of the anterior end of the body. Before the appearance of the first somite, the mesoblast starts to extend into the anterior part of the area opaca. In so doing, it grows forward along the anterolateral borders of the area pellucida but does not invade the portion of the area pellucida that lies directly anterior to the embryo's head. As a result, the mesoblast forms two "horns," one on either side of the mesoderm-free region, which is known as the proamnion. The mesoblastic horns swing around anterior to the proamnion and approach the midline, where they meet and fuse at a rather variable time (usually at or before the 14-somite stage).

Meantime, at the 1-somite stage, the horizontal division of the mesoblast has begun in the portion of the area pellucida anterior to the somite level; by the 7-somite stage, it has extended into the area opaca at all levels (*Duval, 1889*). The separation never becomes altogether complete, in that the somatic and splanchnic layers remain mutually continuous at their distal limits.

Together with the epiblast, the somatic mesoblast constitutes the somatopleure, and the hypoblast and splanchnic mesoblast make up the splanchnopleure; the space between the somatopleure and the splanchnopleure is the coelom. Those portions of the somatopleure, splanchnopleure, and coelom that become incorporated into the body lie medially and are designated "embryonic." They are continuous distally with the "extraembryonic" portions destined to give rise to the membranes. The amnion and the chorion are derived from the extraembryonic somatopleure, and the yolk sac develops from the extraembryonic splanchnopleure. The allantois arises from tissue that is transferred from the yolk sac to the hind-gut; it probably originates in an extraembryonic position, but it grows out into the extraembryonic coelom as a diverticulum of the intestine.

Eventually, the body of the embryo becomes detached from the extraembryonic tissues, except in a very small region where the body wall and the intestine remain open. The opening, or umbilicus, is surrounded by a double-walled tube. The outer, somatopleuric tube, or somatic stalk, connects embryo and amnion, and the inner, splanchnopleuric tube, or yolk stalk, connects embryo and yolk sac. The somatic stalk also encloses the allantoic stalk, which lies directly posterior to the yolk stalk.

## THE YOLK SAC

The yolk sac, which eventually surrounds the yolk completely, is very well vascularized. This characteristic is consistent with the yolk sac's functions of manufacturing blood and of absorbing nutrients from the yolk and



transferring them to the embryo. The yolk sac is thick, opaque, and covered on its inner surface with extensive villus-like projections and corrugations running in a generally meridional direction and containing a multitude of blood vessels and capillaries.

Throughout incubation, the yolk sac is in a continual stage of change. Its constituent germ layers are different at the beginning and the end of its formative period. Even before it has completed its growth, it begins to contract, conforming to the diminished volume of its contents; but as it shrinks, it continues to develop in depth rather than in extent. Its vascular system undergoes a definite structural evolution, both grossly and histologically, and the course of blood circulation through its vessels is radically altered. Nor does its history terminate at hatching, for the remnant of the sac, with its contained yolk, is drawn into the abdominal cavity and gradually disappears during early postembryonic life.

### The Growth of the Yolk Sac

During the period when it is spreading over the surface of the yolk, the yolk sac consists of a medial, vascularized region known as the area vasculosa and a peripheral, nonvascular region termed the area vitellina. The latter is in turn composed of two concentric zones, of which the more medial is the area vitellina interna and the more peripheral is the area vitellina externa. As the area vitellina grows over the yolk, the area vasculosa also increases in size, invading the area vitellina; as a result, the latter eventually disappears completely, and the entire yolk sac is vascularized.

The area vitellina can be traced back to a somewhat earlier stage than the area vasculosa. Its history begins as a continuation of the history of the germ wall and the zone of extension, which together, it will be recalled, make up the area opaca of the early blastoderm (see Chapter 3). A surface view of the area opaca in the hen's egg incubated about 16 hours is given in Fig. 382-A. The area vitellina interna corresponds to the region of the germ wall, where, beneath the superficial layer of ectoderm, there are several irregular layers of cells. These are considered as endodermal because they are continuous with the single layer of flat cells making up the endoderm of the area pellucida. The area vitellina externa represents the zone of extension. In this region, the endodermal cells are dispersed among spheres of yolk and are not clearly delimited. The ectoderm of the area vitellina is continuous with the ectoderm of the area pellucida. At the periphery of the area vitellina externa, according to Duval (1884*c*, 1884*e*), the ectodermal layer projects far beyond the endodermal; but Rauber (1876*b*, *p.* 7), Disse (1879), Virchow (1891), and Dubuisson (1906) stated that only a few, if any, ectodermal cells extend past the outer limits of the endoderm. It is clear from the foregoing that the area vitellina contains only ectoderm and endoderm and has no mesodermal component; as soon



as it is invaded by mesoderm, it is transformed into area vasculosa. The endoderm will form a permanent part of the yolk sac, but the ectoderm (like the somatic mesoderm) is actually part of the chorion, as becomes more apparent when the allantois grows into the extraembryonic coelom.

The area vasculosa, then, is composed of endoderm and also of splanchnic mesoderm, within which blood vessels and connective tissue differentiate. It is difficult to designate a particular time or event as marking the initial development of the area vasculosa. In a sense, the area vasculosa comes into being with the appearance of the first blood islands of Wolff

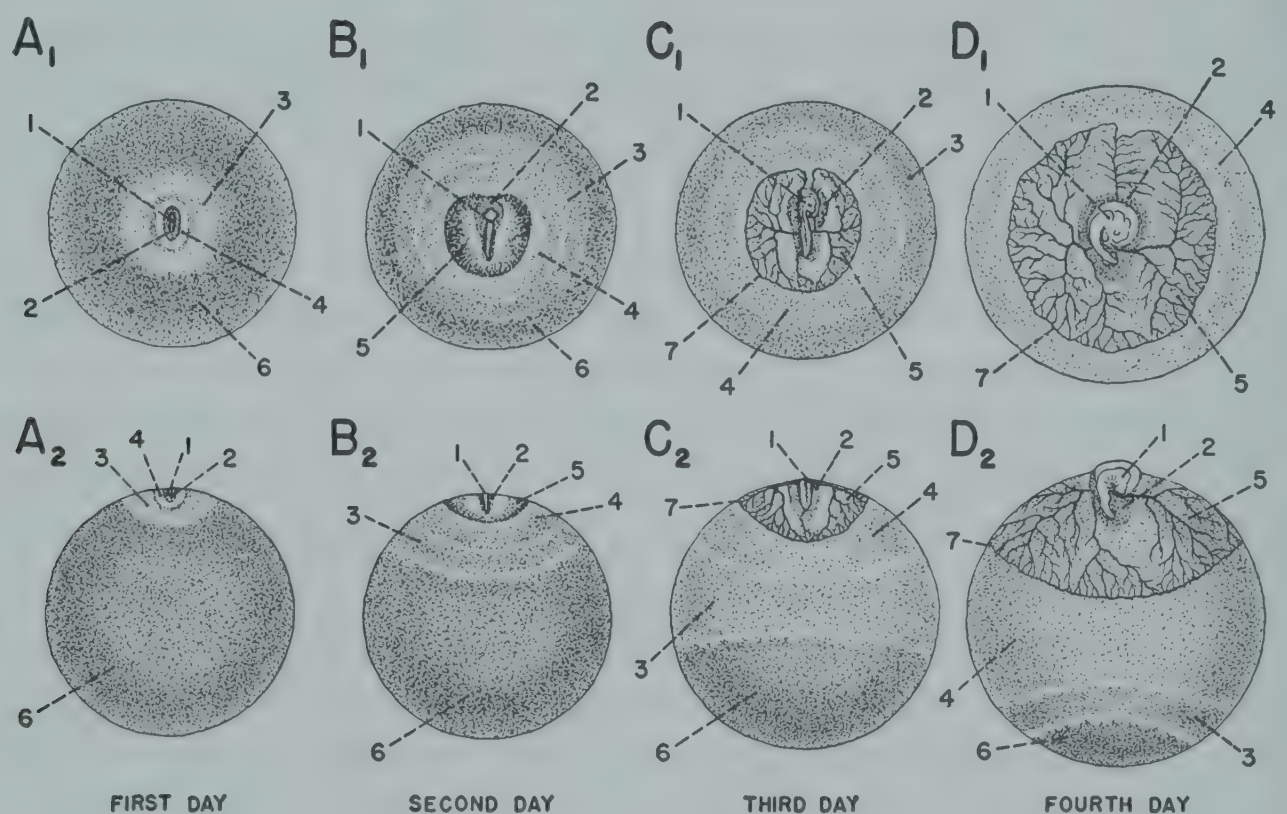


Fig. 382. Successive stages in the growth of the yolk sac over the surface of the yolk during the first 4 days of the chick's development. (Redrawn with modifications after Duval, 1889.)

A's, first day; B's, second day; C's, third day; D's, fourth day. All  $\times 0.66$ .

1, embryo; 2, area pellucida; 3, area vitellina externa; 4, area vitellina interna; 5, area vasculosa; 6, yolk; 7, sinus terminalis.

(the primordia of blood cells and blood vessels) in the posterior part of the area opaca during head-process or early somite stages. Following the same pattern of extension as the expanding mesodermal layer, from which they are derived, the blood islands spread anteriorly and laterally in the area opaca. Before the horns of the mesoderm meet in front of the embryo, the process of vascularization is well along in the area pellucida which constitutes the most proximal part of the area vasculosa for some time (*Giacomini, 1930b*). The area vasculosa is now easily recognizable as a mottled, sharply demarcated, almost circular region fanning out from the embryo in all directions except anteriorly (cf. Fig. 382-B). The anterior gap is bridged at the end of the chick's second incubation day or the beginning



of the third day (*Popoff*, 1894), and the embryo is now completely surrounded by the area vasculosa.

With the peripheral margin of the area vitellina externa acting as its leading edge, the yolk sac progresses over the yolk, passing the equator of the latter (cf. Fig. 382-C) during the chick's third day (*Duval*, 1884*e*, 1889) or fourth day of incubation (*Rauber*, 1876*b*). At the end of the fourth day (Fig. 382-D), the edge of the yolk sac may still be some distance from the distal pole of the yolk (*Duval*, 1889) or may have already reached it (*Grodzinski*, 1934*b*); according to *Rauber* (1876*b*), the yolk is not entirely surrounded until the end of the fifth day. Although virtually enclosing the yolk at this early stage, the yolk sac is still open distally, where there is a small circular aperture known as the yolk sac umbilicus (cf. Fig. 384-C).

During the third to fifth days of incubation, the area vitellina extends itself centrifugally at the average rate of 2.6 to 2.8 mm. per day, as shown by the movement of Nile blue sulfate marks placed on the ectoderm. Its surface area continues to increase until the sixth day. Not all of this expansion is due to growth, however, for the influx of water from the albumen to the yolk causes the latter almost to double in volume during the same period (*Baer*, 1828*b*; *Virchow*, 1891; *Grodzinski*, 1934*b*). It has been suggested that water is withdrawn from the albumen by the vessels at the periphery of the area vasculosa (*Remotti*, 1930*a*) and filtered into the yolk by the vessels of the area pellucida (*Giacomini*, 1930*a*, 1930*b*). An incidental result of the enlargement of the yolk is the rupture of the vitelline membrane, which gives way over the embryo's body on the third day. According to *Duval* (1884*e*), the vitelline membrane then slips down (or is pulled by the growing yolk sac) toward the yolk sac umbilicus, where it remains intact as a partition between yolk and albumen. *Spampani* (1905), however, stated that the vitelline membrane is rapidly resorbed and that yolk and albumen in the region of the yolk sac umbilicus are separated by an extension of the chorion.

It is possible that there is a slight continued narrowing of the yolk sac umbilicus until the chick's twelfth incubation day (*Duval*, 1884*e*). Despite its continued peripheral extension, the area vitellina nevertheless begins to decrease in total area on the seventh day because of the inroads made upon it medially by the area vasculosa; and it has all but disappeared by the fourteenth day (*Grodzinski*, 1934*b*). It is of interest to note that the area vitellina may continue its growth after the death of the embryo, and that it survives longer than the area vasculosa (*Aliberti*, 1933), which therefore is less autonomous (*Remotti*, 1933*b*).

The growth of the area vasculosa is vigorous from the beginning. Its increase in size between the chick's 26- and 84-hour stages can be seen by comparing Fig. 382-B, C, and D; and its encroachment upon the area



vitellina is depicted diagrammatically in Fig. 383. During the period from the third to the fifth incubation days, when it is growing most rapidly, the area vasculosa invades the area vitellina at the rate of 6.0 to 7.4 mm. per day; thus Nile blue sulfate marks placed on the vitelline ectoderm during the third day are soon overtaken by the margin of the area vasculosa. It appears that the marginal portion of the area vasculosa is mainly responsible for extension, since marking experiments also show that the central portion grows no more quickly than the area vitellina (Grodzinski, 1934*b*). It is into the marginal portion that finely divided tumor tissue becomes incorporated after being injected into the yolk. The tumor tissue, vascularized by the yolk sac vessels, is carried by the growing edge of the

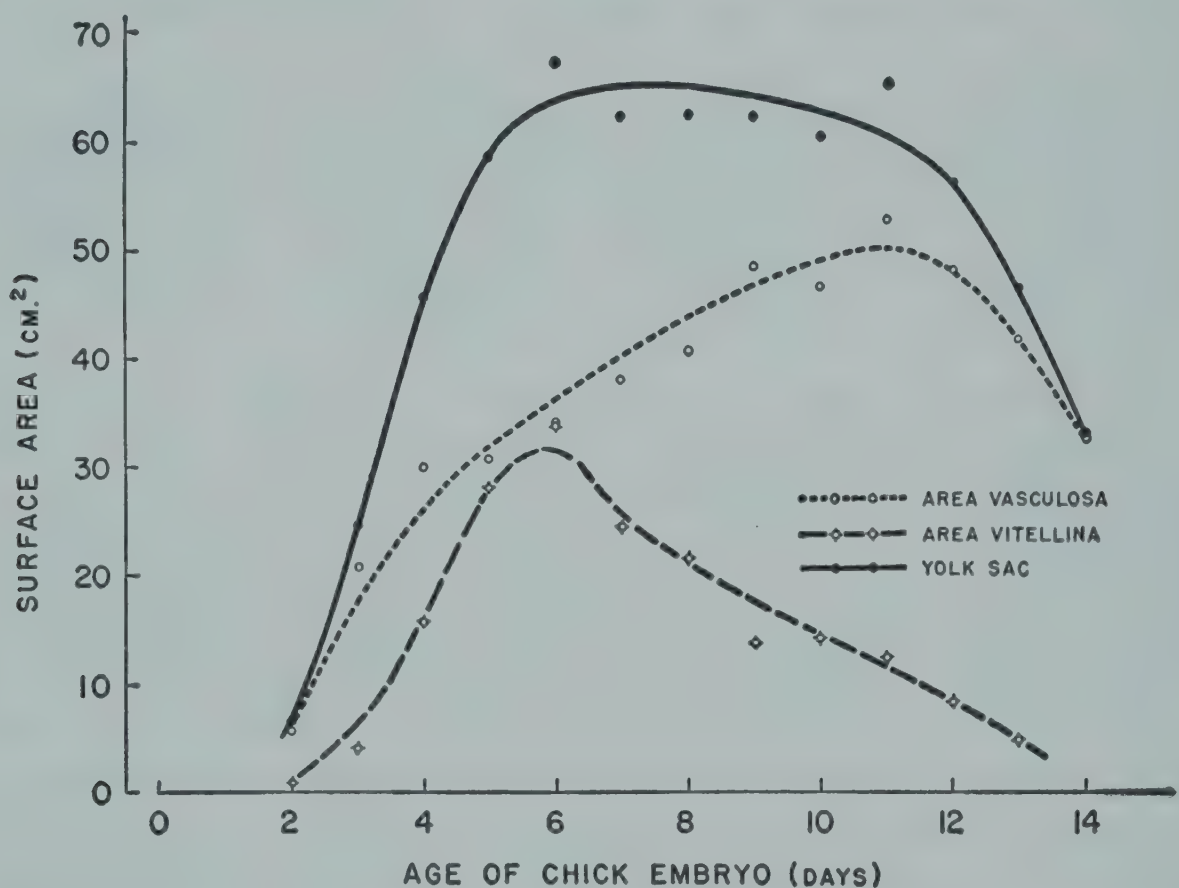


Fig. 383. Changes in the surface area of the yolk sac and its two main zones, the area vitellina and the area vasculosa, in the chicken egg during the first 2 weeks of incubation. (Redrawn with modifications after Grodzinski, 1934*b*.)

area vasculosa to the distal pole of the yolk sac (Taylor, Thacker, and Pennington, 1942; Taylor, Hungate, and Taylor, 1943; Hungate, Taylor, and Thompson, 1944).

When the embryo is killed by various methods on the first to fifth day of incubation, the area vasculosa may not only survive but may also continue to grow for a time (Remotti, 1933*b*; Grodzinski, 1934*b*). Its growth rate, however, is less than half normal; hence it can be inferred that the circulation of the blood stimulates the extension of the area vasculosa (Grodzinski, 1934*b*). The amount of oxygen available also influences the early growth of the area vasculosa, which is underdeveloped when the egg is incubated in rarefied air (16 to 17 cm. of mercury) and overdeveloped when the atmosphere is hyperoxygenated (Giacomini, 1894).



The area vasculosa passes the equator of the yolk between the chick's fifth and seventh incubation days (*Remotti, 1927b*), as may be seen in Fig. 384-A to C. It continues to grow until the fourteenth or fifteenth day (*Grodzinski, 1934b*). By this time, it has gained the margin of the yolk sac umbilicus (*Duval, 1884e*), so that the area vitellina disappears (Fig. 385).

At some time between the chick's sixteenth and nineteenth days of incubation, the yolk sac umbilicus closes (*Virchow, 1891; Spampani, 1905*).

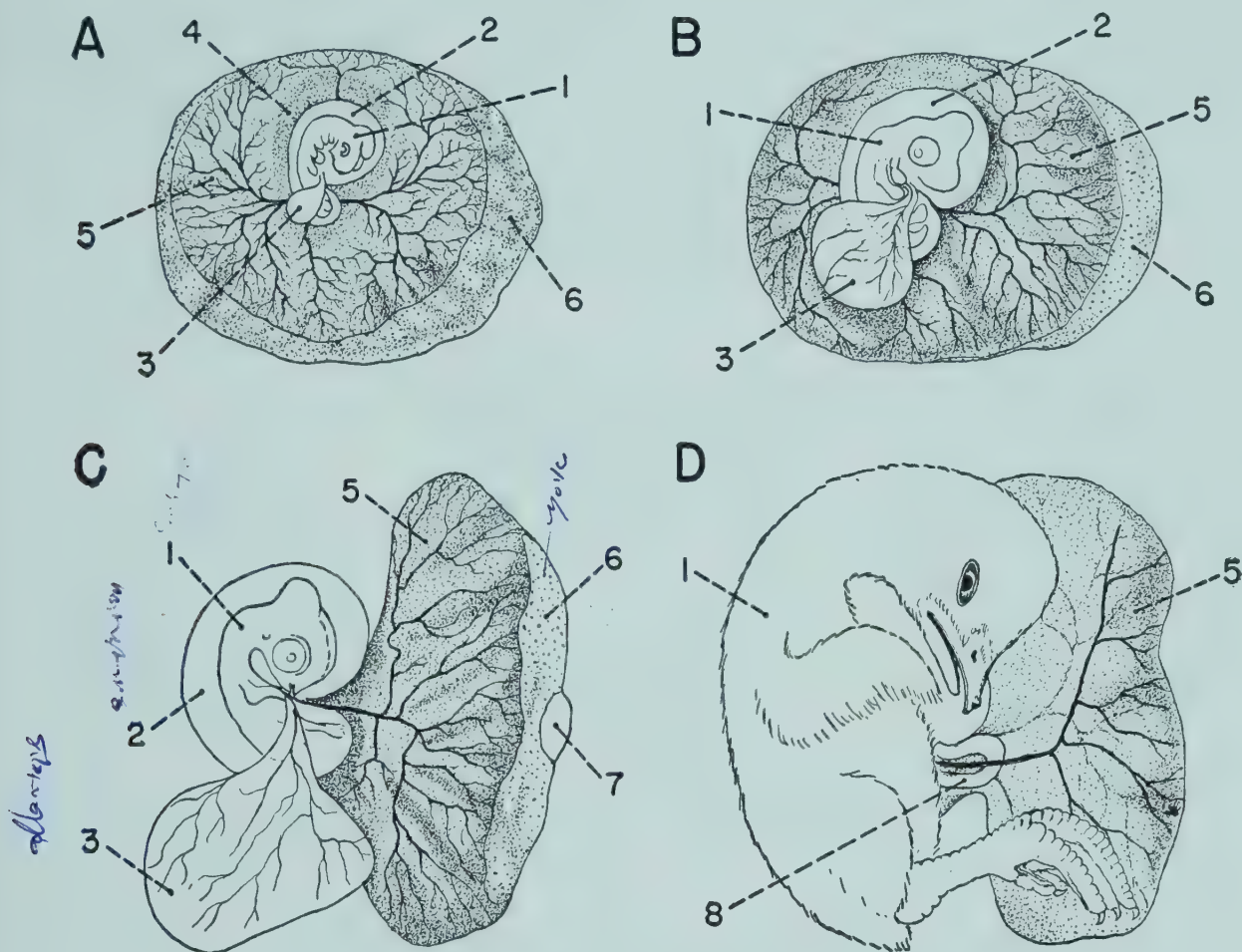


Fig. 384. The gross appearance of the yolk sac of the developing chick embryo at various ages from 4.5 to 19 days. (Redrawn with modifications after *Duval, 1889*.)

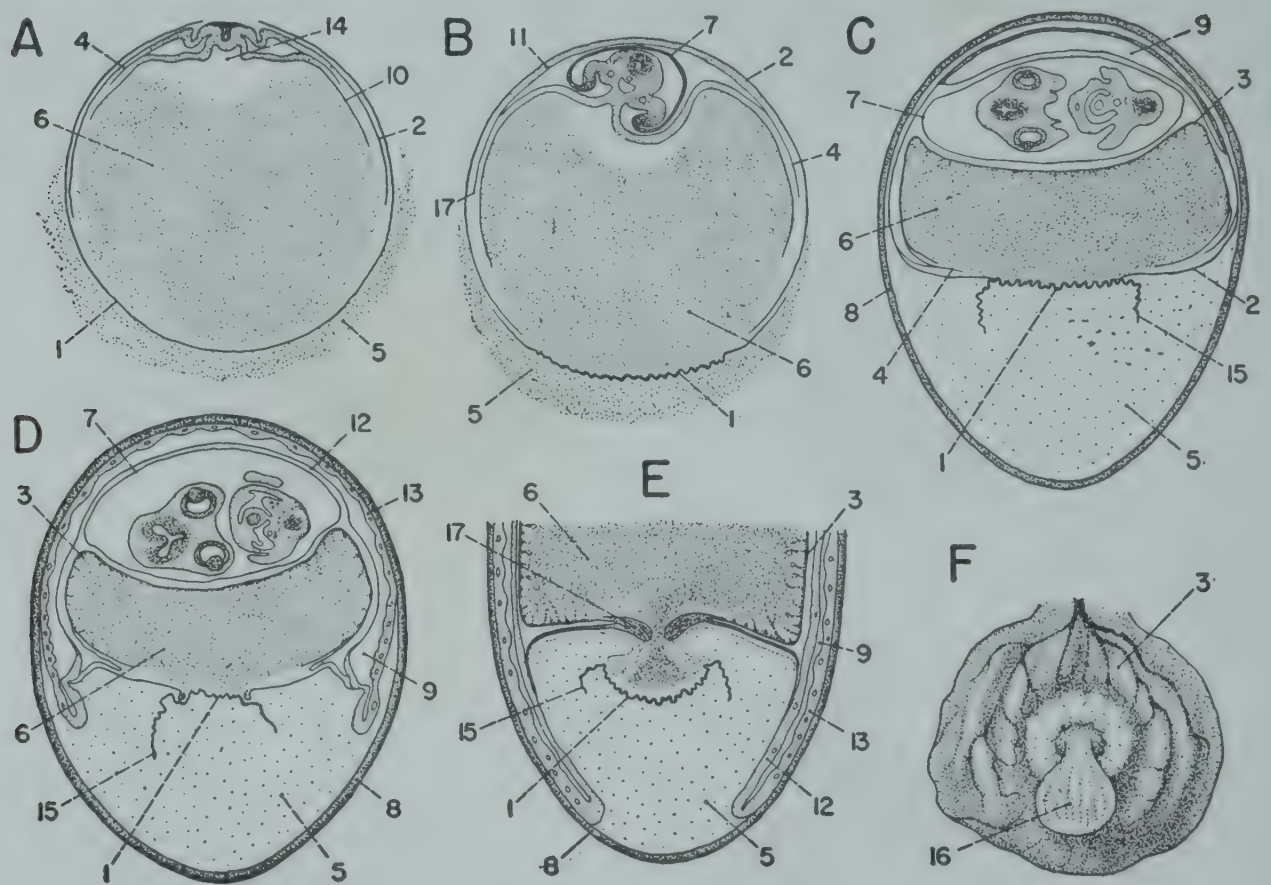
A, during the fifth day; B, during the sixth day; C, during the seventh day, as seen when the embryo and its appendages are removed from the shell and immersed in water; D, during the nineteenth day (the amnion and allantois have been removed). Both  $\times 0.66$ .

1, embryo; 2, amniotic sac; 3, allantois; 4, area pellucida; 5, area vasculosa; 6, area vitellina; 7, yolk sac umbilicus; 8, loop of intestine protruding from abdomen into the umbilical cord.

The ectoderm surrounding the opening has become everted by the eighth or tenth day and has grown down into the albumen rather than across the vitelline umbilicus (Fig. 385-F). A small amount of yolk may have been herniated through the umbilicus into the albumen (*Duval, 1884e; Fülleborn, 1895; Creighton, 1899; Spampani, 1905*), with which it has not mingled because of the presence either of chorionic tissue (*Spampani, 1905*) or of a wrinkled remnant of the vitelline membrane (*Duval, 1884e*). This herniated yolk, if present, is now drawn back inside the yolk sac as



the vitelline umbilicus closes. Closure is effected by the slow centripetal growth and fusion of the mesodermal components of yolk sac and chorion (Spampani, 1905). The mesoderm filling the umbilical aperture is a type of scar tissue (Duval, 1884e; Virchow, 1891), full of blood vessels and the remnants of blood vessels (Spampani, 1905).



**Fig. 385.** A semischematic representation of the development of the yolk sac and vitelline membrane in the chick embryo. (Redrawn with modifications after Duval, 1884e.)

A, transverse section through embryo and yolk, at the beginning of the third incubation day; B, same section at 3.5 days; C, section through the whole egg, transverse to the long axis of the embryo, at 5 to 6 days; D, same section, 8 to 10 days; E, same section, omitting the region of the embryo, at 14 to 15 days; F, yolk sac at 16 days, showing everted ectoderm which has grown down into the albumen rather than across the vitelline umbilicus.

1, vitelline membrane; 2, ectoderm; 3, yolk sac; 4, mesoderm; 5, albumen; 6, yolk; 7, amnion; 8, shell; 9, allantoic cavity; 10, entoderm; 11, chorion; 12, inner allantoic wall; 13, outer allantoic wall; 14, primitive gut; 15, free border of vitelline membrane; 16, everted ectoderm; 17, terminal ridge of mesoderm.

Paradoxically, the area vasculosa starts to decrease in absolute surface extent while it is still growing (cf. Fig. 383). This decrease in the absolute size of the area vasculosa begins on the twelfth incubation day and is accompanied by a similar decrease in the size of the entire yolk sac. Both these changes are related to the absorption of yolk. On the seventh day, the amount of yolk begins a sharp decline that continues until hatching. The yolk sac, however, is still extending itself and shows only a very slight diminution in surface area until the twelfth day; at this time, having com-



pletely surrounded the yolk, it begins to shrink rapidly as it accommodates itself to the continually smaller volume of its contents (cf. Fig. 383).

When the growth of the yolk sac is expressed in terms of weight rather than surface area, a somewhat different picture is obtained. The weight of the membrane shows an uninterrupted increase until the sixteenth day (Fig. 386), the rate of increase being most rapid up to the sixth day and somewhat more gradual thereafter (Byerly, 1932; Needham, 1932a). The continued gain in weight after the onset of shrinkage in surface area reflects not only the presence of large amounts of absorbed yolk in the yolk sac cells, but also structural changes in the yolk sac wall. Although the yolk

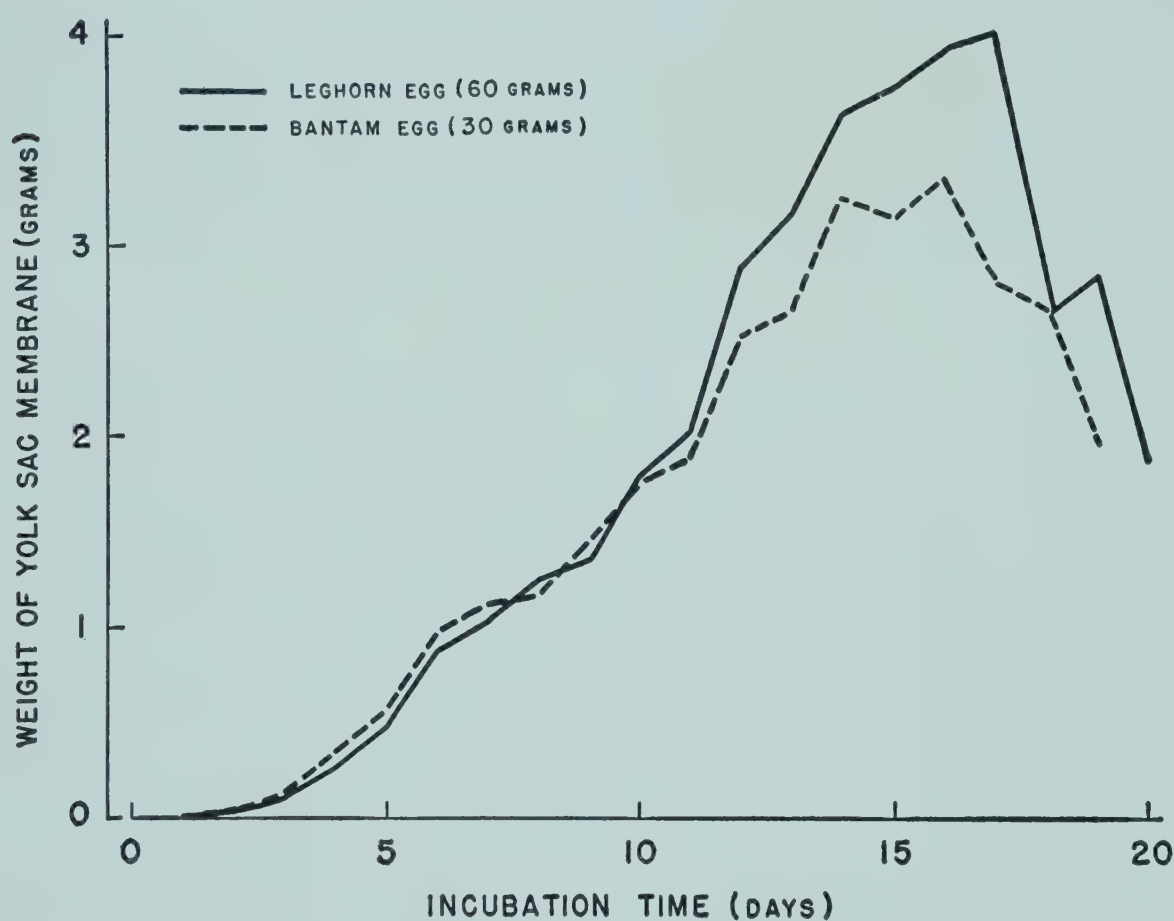


Fig. 386. Growth of the yolk sac membrane in large (Leghorn) and small (Bantam) chicken eggs (*Gallus gallus*) as shown by its progressive increase in weight during the first 16 days of incubation. (Plotted after the data of Byerly, 1932.)

sac is of approximately constant thickness until it has completed the process of yolk enclosure, it then becomes much thicker and heavier as the radial folds on its inner surface extend continually farther out into the substance of the yolk. After the sixteenth day, the membrane shows a moderate weight loss prior to its retraction into the embryo's body.

The early, most rapid period of growth is associated with a relatively high respiratory quotient. The latter is 0.9 from the third to the sixth day but only 0.6 on the eighth day and thereafter (Needham, 1932b, 1933). The high initial value appears to reflect the formation of the yolk sac rather than its function of distributing material to the embryo and the other membranes (Needham, 1933), since calculations show that most of the



yolk absorbed during the first week is used for the construction of the yolk sac itself. The low respiratory quotient during the last 2 weeks of incubation has not been explained satisfactorily. Oxygen consumption by the yolk sac also is high (320 cu. mm/gm/hr) during the entire growth period and then begins to decline after the thirteenth or fifteenth day, so that it has diminished by about 40 per cent by hatching time (*Needham, 1932a*). Calculations (in which the allantois was included) have shown that the efficiency with which inert material is transformed into living cells is 90 to 97 per cent before the twelfth day, drops to 72 per cent by the fourteenth day, and then rapidly falls to zero, because respiration continues whereas weight increments soon cease (*Needham, 1932a*).

It is of interest to note in passing that the (wet) weight of the yolk sac, in terms of percentage of the living matter in the egg, decreases from 75 per cent on the third day to 15 per cent on the tenth day, remains constant at this value until the sixteenth day, and then undergoes a further decline to about 7 per cent on the nineteenth day (*Byerly, 1932*).

### The Yolk Stalk

The yolk stalk is the peduncle which attaches the yolk sac to the intestine and by means of which the vitelline blood vessels enter the embryo. The greater part of the process of yolk stalk formation is simultaneous with and complementary to the closure of the mid-gut, which is accomplished by the backward migration of the anterior intestinal portal and the forward migration of the posterior intestinal portal. By the beginning of the chick's fifth day of incubation, the opening from the gut to the yolk sac has been reduced to a short, narrow slit. Soon the connection assumes a more tubular form. By the end of the sixth day, the yolk stalk, with a loop of attached intestine, is enclosed within the outer or somatic stalk, which is the neck of the amnion. The protruding intestine is not retracted into the body until the eighteenth or nineteenth day (*Duval, 1889; Virchow, 1891*). According to Virchow (1891), the yolk stalk grows to a maximum length of 4.0 mm. on the sixteenth day and then becomes shorter; Maumus (1902) found it to be 3.0 mm. long on the twentieth day. Late in incubation, the vitelline blood vessels are suspended from the yolk stalk in mesenteries (*Virchow, 1891; Giacomini, 1893*).

During the last week of the chick's incubation period, the wall of the yolk stalk develops a structural similarity to the intestinal wall. Longitudinal bundles of smooth muscle fibers are apparent on the fourteenth incubation day. As the fibers increase in number they form an uninterrupted layer. The connective tissue also increases markedly in density and amount and is in part circular in arrangement. The mucosal layer is disposed in longitudinal folds and contains recesses which bear a certain superficial resemblance to simple tubular glands. The cells of the lining epithelium



form a single layer; on the nineteenth day, they are cuboidal in form at the midlevel of the yolk stalk and resemble intestinal epithelial cells at its proximal end (*Virchow, 1891*).

### Spatial Relationships between the Yolk Sac and the Embryo

During the course of incubation, the yolk sac and the embryo do not remain in the same position, either relative to each other or to the rest of the structures within the egg. The changing spatial relationships between the yolk sac and the chick embryo have been well described by Kuo (1932c).

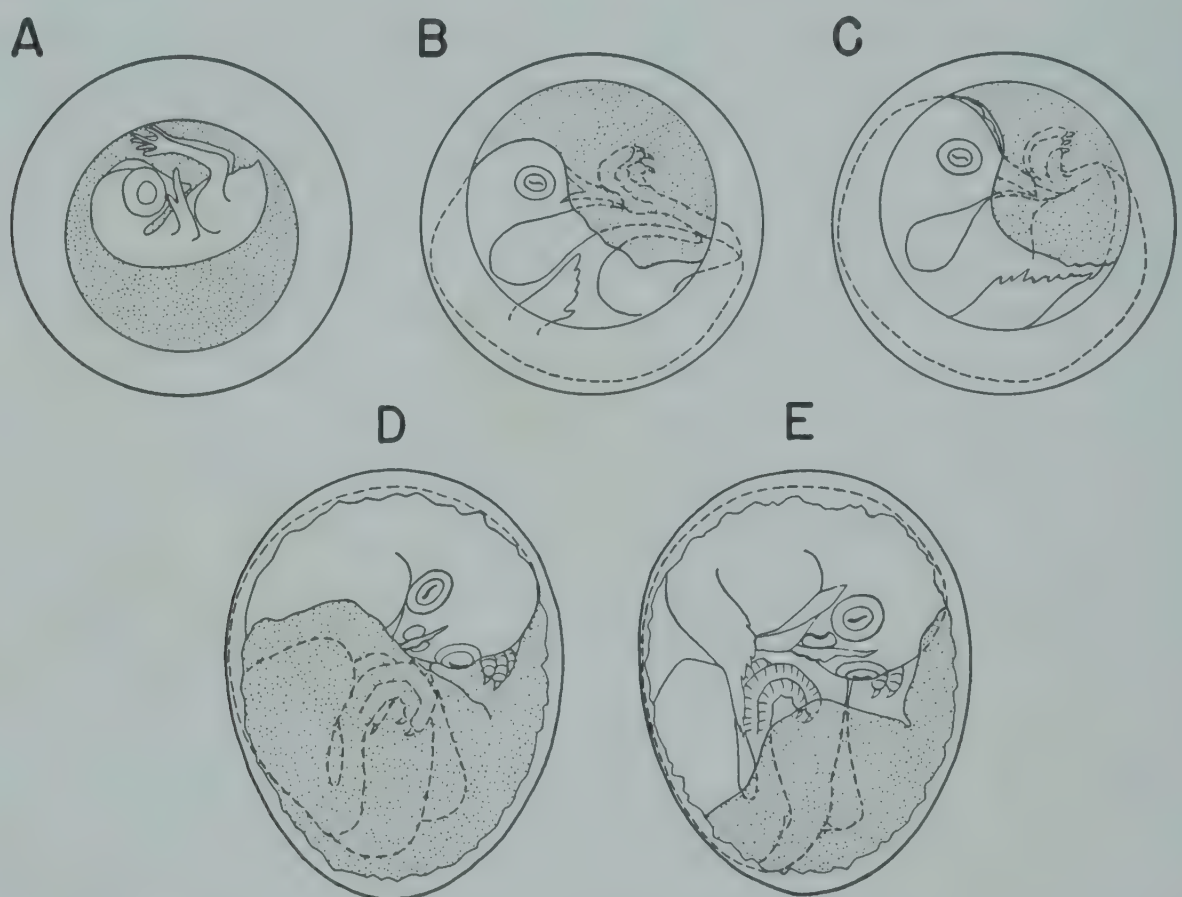
During the first 4 days of incubation, the embryo is found at the pole of the yolk which is uppermost in an egg lying with its long axis horizontal. The yolk itself, however, does not remain stationary throughout this period. By the end of the second day of incubation, the albumen has increased in specific gravity (through water loss) to such an extent that the less dense yolk rises and comes into virtual contact with the inner shell membrane (*Féré, 1897; Remotti, 1930a*). By the fifth day, the embryo has turned on its left side. It is now much heavier than before and lies in a large depression in the area pellucida, which, viewed from below, appears as a thin vascular membrane stretched taut over the amnion with its contained embryo (*Giacomini, 1930b*). During the period from the fifth to the ninth day, the amnion is contracting vigorously. The tendency of the yolk to rise is now counteracted not only by the continually heavier weight of the embryo's body but also by the contractions of the amnion. Each contraction pushes down the yolk sac, which, at the end of the contraction, immediately expands upward, aided by the resistance of the increasingly dense albumen beneath. The upward expansion is greater on the ventral side of the embryo than on its dorsal side. As a result, the embryo is turned on its back as it sinks farther into the yolk and also is moved into the large end of the egg, where it lies transverse to the egg's long axis (Fig. 387-A). The albumen, meantime, has been collecting in the small end of the egg; by the tenth day, the line of separation between the yolk sac and albumen runs from the lower edge of the air cell to the middle of the sharp end of the egg (*Fülleborn, 1895*).

After the contractions of the amnion cease, the drift of the embryo toward the eggshell on its dorsal side continues, and so also does the tendency of the yolk to react to the increasing weight of the embryo's body by spreading into any available space. Between the tenth and the fourteenth day, the yolk sac slips gradually farther over the ventral surface of the embryo and withdraws from its dorsal surface. By the thirteenth or fourteenth day, therefore, the back of the embryo is close to or touching the inner surface of the shell, and the yolk sac is found on the embryo's left and over its ventral surface, covering the beak, forehead, and legs (Fig. 387-B). The caudal end of the embryo now begins to turn to the left (Fig.



387-C), the neck acting as a pivot, so that the embryo lies in the long axis of the egg (Fig. 387-D) by the seventeenth day (*Baer, 1828b*). With this change in position, the yolk sac slips farther to the embryo's right; but most of it is over the ventral surface, in readiness for retraction into the body cavity.

The yolk sac begins to enter the embryo's body cavity (Fig. 387-E) on or after the nineteenth day (*Baer, 1828b; Virchow, 1891*). It has been suggested that the yolk sac is either impelled through the umbilicus by contractions of amniotic and allantoic muscles (*Virchow, 1891*) or is drawn in by the muscles in the yolk stalk (*Giacomini, 1893*). Kuo (1932c) found,



**Fig. 387.** Semidiagrammatic representation of changes in the relative position of the yolk sac and chick embryo as seen through an opening in the eggshell. The yolk sac is stippled. (Redrawn with modifications after Kuo, 1932c.)

A, 9 to 10 days; B, 13 days; C, 15 days; D, 17 days; E, 19 days. A, B, C, from large end of egg; D, E, viewed from above. All  $\times 0.4$ .

however, that the amniotic musculature has usually degenerated before hatching time, and he was unable to detect any allantoic contractions while the yolk sac is being drawn in; in fact, he noted that tension exerted by the allantois tends to pull the yolk sac out. His observations indicated that it is chiefly the movement of the abdominal muscles that causes the retraction of the yolk sac. As the abdominal cavity distends, part of the yolk sac enters it, only to be forced out again when the body cavity contracts; but usually less is forced out than enters, so that eventually the entire yolk sac is enclosed. When pulmonary respiration begins, the respiratory movements are of considerable assistance.



### The Yolk Sac Endoderm

The extraembryonic endoderm, which lines the internal surface of the yolk sac, is derived almost entirely from the deeper (subectodermal) part of the germ wall and the zone of extension. Its most medial portion, however, consists of the endoderm of the area pellucida, which is continuous with the endoderm of the gut.

The yolk sac endoderm passes through several developmental stages and finally differentiates into a single layer of cylindrical epithelial cells. This epithelial layer forms an investment for the yolk sac blood vessels and is in direct contact with the yolk. Its function is the absorption and partial digestion of yolk, which, after chemical transformation, is transferred to the blood vessels and carried to the embryo. The absorptive surface of the epithelial layer is greatly increased by the formation of folds or plicae that project into the yolk.

### *The Differentiation of Vitelline Epithelium*

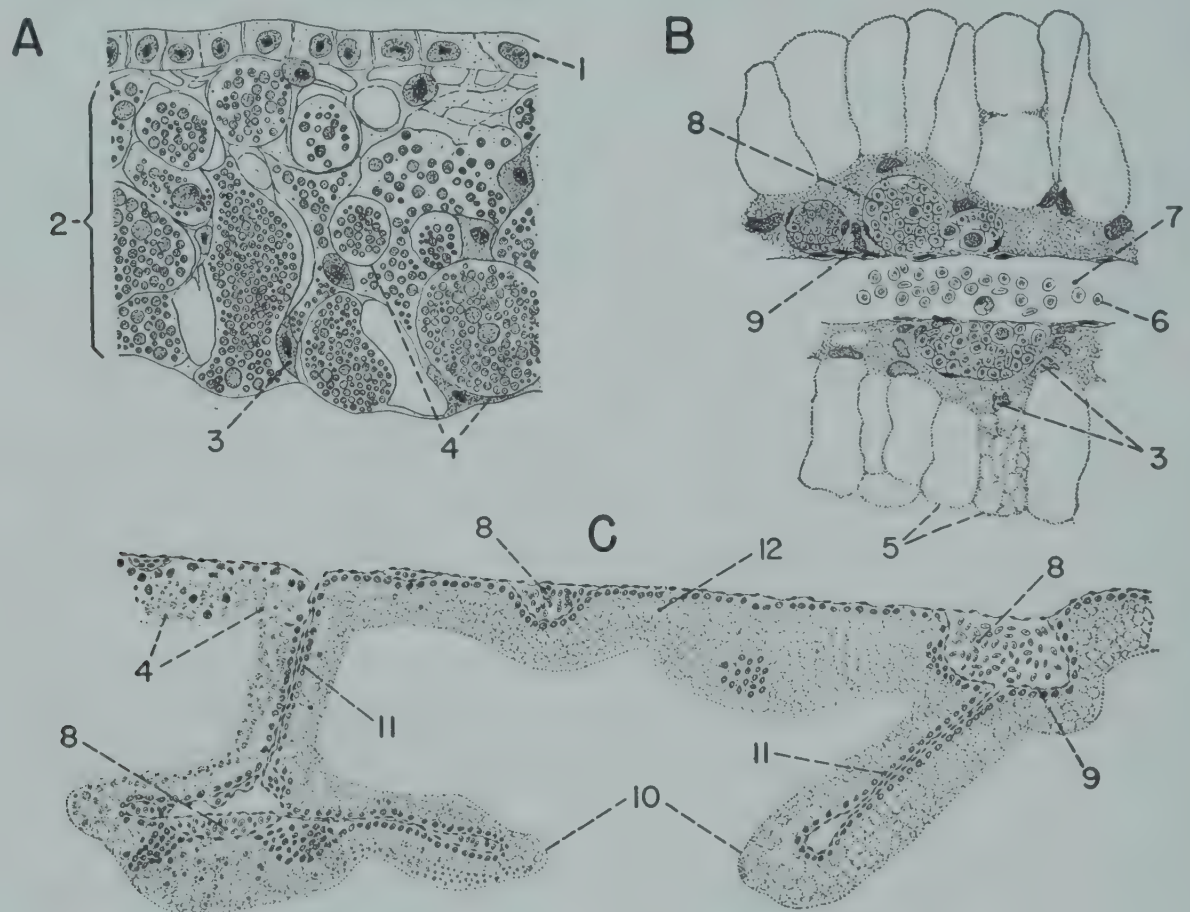
Many early descriptions of the initial development of extraembryonic endoderm are marred by the fallacy that white yolk spheres in the region of the germ wall are cellular in nature, or are directly transformed into cells (*Remak, 1855; His, 1868; Goette, 1874*). Although Kölliker (1875) distinguished the cellular germ wall from the noncellular white yolk and gave a brief but accurate account of the origin of yolk sac endoderm, the observations made by Virchow (1875*b*, 1891) are the most complete in the literature.

As Virchow (1875*a*, 1875*b*, 1891) showed, the essential feature of the development of yolk sac endoderm is the gradual evolution of a single-layered epithelium of large cylindrical elements from very irregularly arranged cells. Successive stages in this process can be seen from the periphery to the proximal part of the yolk sac as the latter grows centrifugally. The most immature stages are found in the distal portion of the yolk sac, where new cells are proliferated; the fully differentiated condition exists medially, where the cells are oldest; and the transitional phases can be seen in the intermediate regions. It follows that several concentric zones are recognizable in the extraembryonic endoderm until it consists of an epithelium in its entirety. At the periphery, there is a narrow, tapering zone of flat cells extending practically to the edge of the overlying ectoderm, and medial to these cells is a region where nuclei seem to be scattered sparsely in the yolk and cell boundaries are indistinguishable. These two zones make up the endoderm of the area vitellina externa. Throughout the area vitellina interna, and extending beneath the mesoderm at the margin of the area vasculosa, is a zone of irregularly stratified cells with many yolk inclusions. In a still more medial position, approximately coextensive with the area



vasculosa, is the single-layered cylindrical epithelium, which in turn gives way to the flat endodermal epithelium of the area pellucida. The zone of cylindrical epithelium widens at the expense of the stratified zone, and the latter extends itself at the expense of the most distal cells.

These cells, which are the youngest, are frequently distorted in shape by extracellular and intracellular yolk spheres. According to Virchow (1891), their normal shape is round in surface view and round or spindle-shaped in section; they each contain a centrally placed nucleus and finely reticular cytoplasm. Mitotic figures are very numerous in this zone.



**Fig. 388.** Stages in the development of the endodermal epithelium of the yolk sac of the chick, semidiagrammatic representations. (Redrawn with modifications from Grodzinski, 1930; B and C, from Dubuisson, 1906.)

A, section through the area vitellina at the 2-day stage ( $\times 500$ ); B, section along the axis of a fold in the wall of the area vasculosa of the 8-day yolk sac ( $\times 200$ ); C, section of the wall of the 8-day yolk sac near the outer edge of the area vasculosa ( $\times 100$ ).

1, ectoderm; 2, endodermal layer; 3, nucleus of endodermal cell; 4, yolk sphere; 5, endodermal cell; 6, blood cell; 7, lumen of longitudinally sectioned blood vessel; 8, blood vessel in cross section; 9, endothelial nucleus; 10, fold; 11, axial connective tissue of fold; 12, vacuole.

The transition to the more medial stratified zone is gradual. As the cells take up yolk, they increase in size and become crowded into three or four irregular layers. This zone is recognizable at primitive streak stages, when it is about twelve cells wide and constitutes the conspicuous inner boundary of the area opaca. By the end of the chick's second day of incubation it has widened greatly and has moved distal to the area vasculosa to give rise to the area vitellina interna (Fig. 388-A). While this area spreads centrifugally



by the addition of cells from the peripheral zone, the cells at its medial border differentiate as cylindrical epithelial elements and join the epithelial zone. The stratified zone is completely transformed into epithelium at some time after the chick's twelfth incubation day, when it can still be seen under the mesodermal elements near the edge of the area vasculosa (*Virchow, 1891*).

The cells of the stratified layer are more or less round in surface view on the second and third days, but become polygonal as they are packed more closely together. They contain yolk spheres which are somewhat smaller than those in the more peripheral cells but larger than those in the one-layered epithelium. At the end of the third day the yolk inclusions are smaller than on the second day and so numerous that cell membranes are difficult to distinguish.

The stratified zone passes gradually into the medial epithelial zone. The latter may be said to exist already during late primitive streak stages, when the transition from the endoderm of the germ wall to the flat endoderm of the area pellucida is made by means of a few cells of intermediate form disposed in a single row. The one-layered zone has extended into the area vasculosa by the 5-somite stage and has almost reached the periphery of the latter by the 9-somite stage. Thereafter, as the area vasculosa expands, the single-layered epithelium keeps pace with it (Fig. 388-C), forming a continuous layer beneath all but the marginal blood vessels (*Virchow, 1891*).

At first, the epithelial cells are cuboidal. During the third day they become cylindrical between the blood vessels but remain cuboidal directly beneath them, being especially low under the more prominent vessels (*Virchow, 1875b, 1891*). Soon all the cells become cylindrical. The cell boundaries are distinct, and the free ends of the cells—the ends facing toward the yolk—are rounded. The nuclei are basal in position (*Dubuisson, 1906; Remotti, 1927b*), contain one or two nucleoli, and are often deformed by the pressure of the yolk inclusions in the cytoplasm. After 5 to 7 days of incubation, the epithelial cells are two or three times as high as wide (Fig. 388-B). At this time, they are so closely packed together and so full of yolk inclusions that they vary greatly in appearance, some being cylindrical and others conical, pyriform, or hourglass-shaped. During the latter part of the incubation period, the area pellucida disappears, and the yolk sac endoderm close to the embryo becomes almost as high as it is elsewhere. By the eighteenth day, the epithelial cells have begun to decrease in height throughout the yolk sac (*Virchow, 1891*).

The yolk sac endoderm appears to possess a high degree of autonomy. In yolk sacs that survive the death of the embryo, the endodermal component may live and continue to grow after the vascular tissue has degenerated (*Remotti, 1933b*).



*The Development of Folds*

Folds begin to project from the inner surface of the yolk sac by the middle of the chick's fourth incubation day (Courty, 1848; Dubuisson, 1906). As Remotti (1927*b*) noted, it is not clear whether these structures are formed by the autonomous wrinkling of the yolk sac epithelium, or whether they are secondary to the tendency of the blood vessels to sink or penetrate into the yolk substance. It is obvious that the folds form in intimate relationship with the blood vessels (cf. Fig. 388-C), and that the course of most of them follows the course of vascular channels (Virchow, 1891). Nevertheless, some folds develop independently of blood vessels or contain only very small ones (Dubuisson, 1906; Remotti, 1927*b*). According to Dubuisson (1906), when the stratified cells rearrange themselves into a simple epithelium, the increase in endodermal surface area leads to the formation of folds, into which the splanchnic mesoderm penetrates or is drawn, carrying the blood vessels with it. Whatever the mechanism underlying their initial development, the folds become progressively more complicated in structure during the course of incubation and thereby vastly increase their epithelial surface. As Remotti (1927*b*) remarked, the yolk sac thus manifests a very complex adaptation to its absorptive function, which, of course, resides primarily in the endoderm.

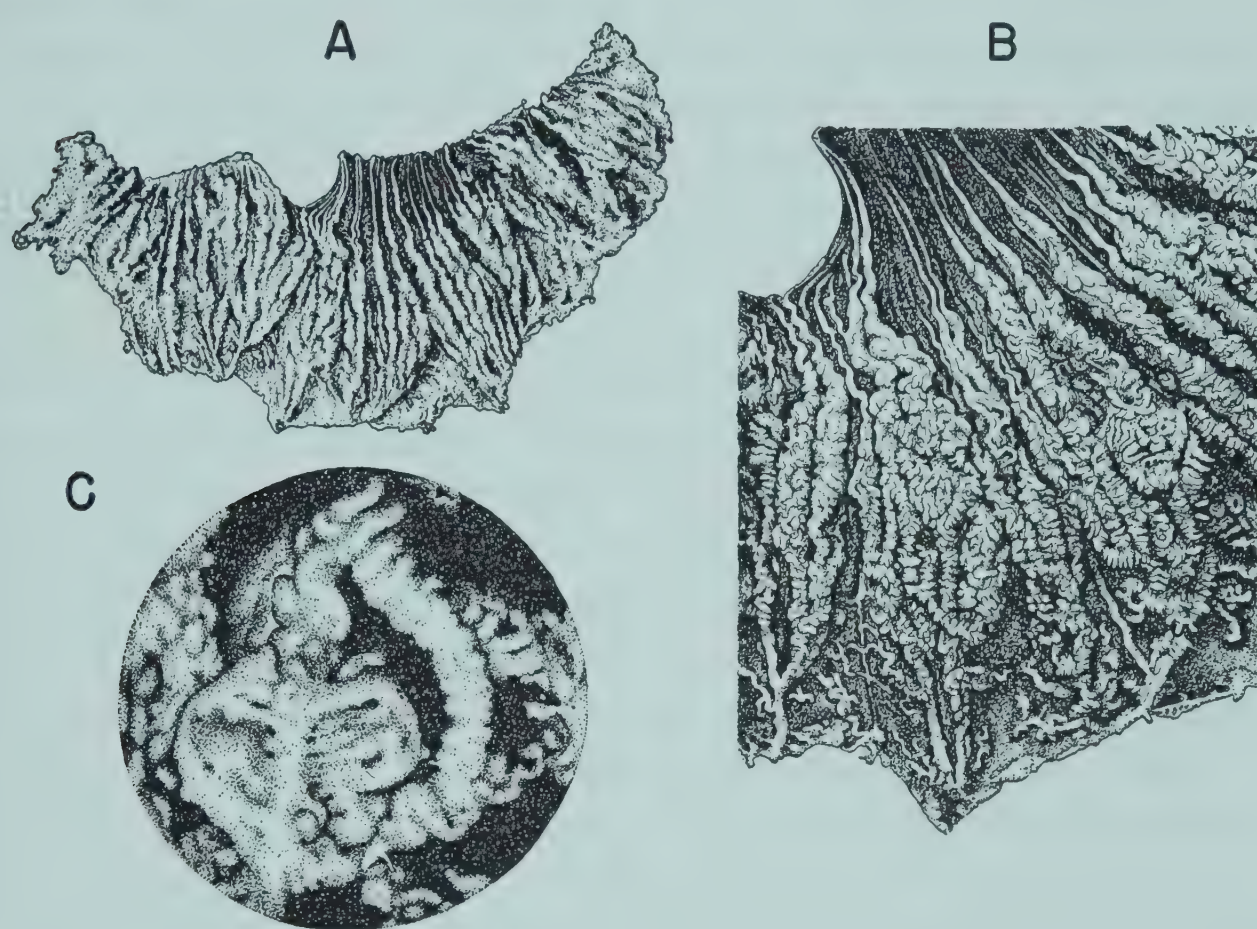
While still low, the folds projecting into the yolk frequently turn and run parallel to the surface of the yolk sac (cf. Fig. 388-C), and consequently they often come in contact with each other and fuse together. In this way, two folds may unite to form a loop hanging into the substance of the yolk (Dubuisson, 1906). Courty (1848), who observed such anastomoses on the fifth day, believed that they are produced by sprouting of the blood vessels within the folds, but, as Dubuisson (1906) stated, union occurs first between the epithelial cells.

Within the period from the fifth to the eighth days, it becomes apparent that the folds are highest in the equatorial region. They run in the meridional direction, bifurcating and branching in conformity with the ramifications of the blood vessels which they contain; they now project much farther into the yolk, as if suspended by mesenteries, and their free edges are sinuous (Fig. 389-A and B). Their axes are occupied by a double layer of splanchnic mesoderm (cf. Fig. 388-C), representing reduplicated connective tissue, against which blood vessels are applied (Dubuisson, 1906; Remotti, 1927*b*).

In the second half of the incubation period, the folds become continually more tortuous and convoluted and project still farther into the yolk, eventually attaining a height of 3 to 7 mm. (Courty, 1848). At the same time, their structure becomes more complex. In general, the change is due to a perforation of the epithelium between the fine capillaries contained



within the folds, so that an intricate lacework is formed (Fig. 389-C). This lacework consists, essentially, of a reticulum of minute epithelial trabeculae enclosing a network of capillaries. Eventually, prominent epithelial cords, which enclose the larger blood vessels, appear to be suspended in the yolk by rows of fine epithelial filaments, each of which contains a capillary (Virchow, 1891; Remotti, 1927*b*). Perhaps the best description in English of the folds in the chick's yolk sac was made by Dalrymple in 1844: "We then see that vessels alone constitute the framework of the undulating folds, the large trunks of which form the base, while innumerable lesser



**Fig. 389.** Gross appearance of the folds on the inner surface of the 12- to 14-day chick embryo's yolk sac. (Redrawn by the author with modifications after Remotti, 1927*b*.)

A, at the 14-day stage, shown at about half natural size; B, a portion of A enlarged to twice natural size; C, from a 12-day yolk sac, enlarged about thirteen times, to show minute collateral folds.

branches dip deeper into the interior of the sac, inosculating repeatedly, and terminating in a series of very tortuous branches that fringe the extreme edge of each plica. Numerous simple loops are observed, shooting from the sides of the larger trunks, and constituting the simplest type of vascular papillae or villosities of mucous surfaces."

### *The Culture of Yolk Sac Endoderm in Vitro*

Yolk sac endoderm from both the area vasculosa and the area vitellina has been successfully grown *in vitro*, but, because of the intense proteolysis associated with cultures derived from the area vasculosa (Thomas, 1933,



1938), studies have been largely confined to those taken from the area vitellina. It is possible to remove the ectoderm before planting the culture (Thomas, 1934a, 1938), so that pure cultures of endoderm may be obtained. When the ectoderm is not removed, ectodermal and endodermal cells grow together in a single layer and are difficult to distinguish, the ectodermal cells being identifiable chiefly by their smaller size and lack of yolk inclusions (Grodzinski, 1930; Thomas, 1933, 1938).

The endodermal cells grow out rapidly as a sheet of epithelium with a sharply defined border. They contain many yolk inclusions, some of which are perhaps secreted by the cells themselves; and their cell membranes are easily demonstrable. A system of radial and circular fibers (collagenous or precollagenous) characteristically develops. With the passage of time, most of the yolk inclusions are digested and the cells begin to spread apart from each other. The separation of the cells begins near the explant and progresses outward, eventually involving the marginal cells (Grodzinski, 1930; Thomas, 1933, 1934a, 1935, 1938).

Growth, as shown by the increase in the area of the culture, is influenced by the composition of the medium and is promoted by the presence of embryo extract, especially in a concentration of 50 per cent (Thomas, 1933, 1934a, 1938). Even in rabbit serum (25 per cent, in Tyrode's solution), with no embryo extract, growth is superior to that of epithelium of any other origin in media containing embryo extract in optimum concentration. Vitelline endoderm also grows in pure Tyrode's solution (Thomas, 1938) and in Locke-Lewis solution (Grodzinski, 1930), although at a retarded rate. According to Grodzinski (1930), the cultures continue to enlarge for twice as long a time (10 days) in media containing plasma as in those containing embryo extract. When the ectoderm is not removed, the size of the cultures in the presence of 20 or 30 per cent embryo extract is no greater than that of pure cultures in rabbit serum alone; hence the ectoderm seems to inhibit the growth potentialities of the endoderm (Thomas, 1938).

Growth (in the presence of embryo extract) begins to decelerate on the fourth or fifth day of culture (Thomas, 1934a, 1938), or may cease entirely at that time (Grodzinski, 1930); in Carrel flasks, it usually continues for 7 days (Thomas, 1934a, 1938). The retardation in growth on the fifth day (in Carrel flasks) coincides with a drop in the pH of the medium (if the latter is not renewed) from 7.7 to 7.55; the final value, on the seventh day, is 7.26. Renewal of the medium at various times from the second to the fourth day prevents the pH from falling below the value of 7.55 by the seventh day (Thomas, 1938).

Multiplication of yolk sac endodermal cells occurs by mitosis only during the first 2 or 3 days of culture, despite the continued increase in area after



this time. Thomas (1938) reported that mitosis is replaced by direct division of the nuclei, which are of very diverse sizes.

The epithelial characteristics of the culture are profoundly modified by the time most of the yolk inclusions are digested and the cells have spread apart. The membrane is now more or less a reticulum, with many lacunae. Elongated cells begin to migrate beyond the no longer sharply demarcated margin of the culture. These cells are typical fibroblasts. Their numbers increase with each subculture. By the sixth passage, all the cells that have not degenerated have dedifferentiated, and the transformation of the culture to a colony of fibroblasts is complete (*Thomas, 1934a, 1935, 1938*).

The physiological properties of fibroblasts derived from yolk sac endoderm were investigated by Thomas (1935, 1938), who stated that the cells reacquire the power of dividing by mitosis but lose the capacity to phagocytose or absorb substances such as India ink and trypan blue. On the other hand, when yolk is added to the culture medium, partially digested fatty inclusions appear in the cells, which, therefore, retain the ability to digest and absorb yolk. Also, they still exhibit the property of secreting a substance that resembles yolk, but only when grown under conditions unfavorable to their multiplication. Fibers are found in the fibroblast cultures, but they differ in appearance from those present in the epithelial cultures.

The growth rate of the fibroblasts is considerably less than that of the epithelial cells (*Thomas, 1934a*). After 6 days, fibroblast cultures grown in the presence of embryo extract (30 per cent) attain one fourth the size of epithelial cultures, or one fifth their size when grown in serum. Growth is not stimulated by increasing the proportion of embryo extract above 30 per cent. The cultures continue to increase in size for 8 days in media containing embryo extract but grow for 10 days (at a greatly reduced rate) in heparinized plasma (*Thomas, 1938*).

It is said that vitelline endoderm cells may transform into histiocytes (macrophages) as well as into fibroblasts. Thomas (1937, 1938) stated that a large epithelial cell may give off histiocytes by direct division until it is entirely consumed in the process. Histiocytes may be formed simultaneously with fibroblasts and can be separated from the latter as a pure culture because, for growth, they require plasma or serum proteins rather than embryo extract. They may be elongated, branched, or round in shape, and they multiply by mitosis. Their phagocytic properties are pronounced, and, unlike vitelline epithelial cells, they engulf erythrocytes. When their growth slows, they secrete small intracellular granules resembling yolk.

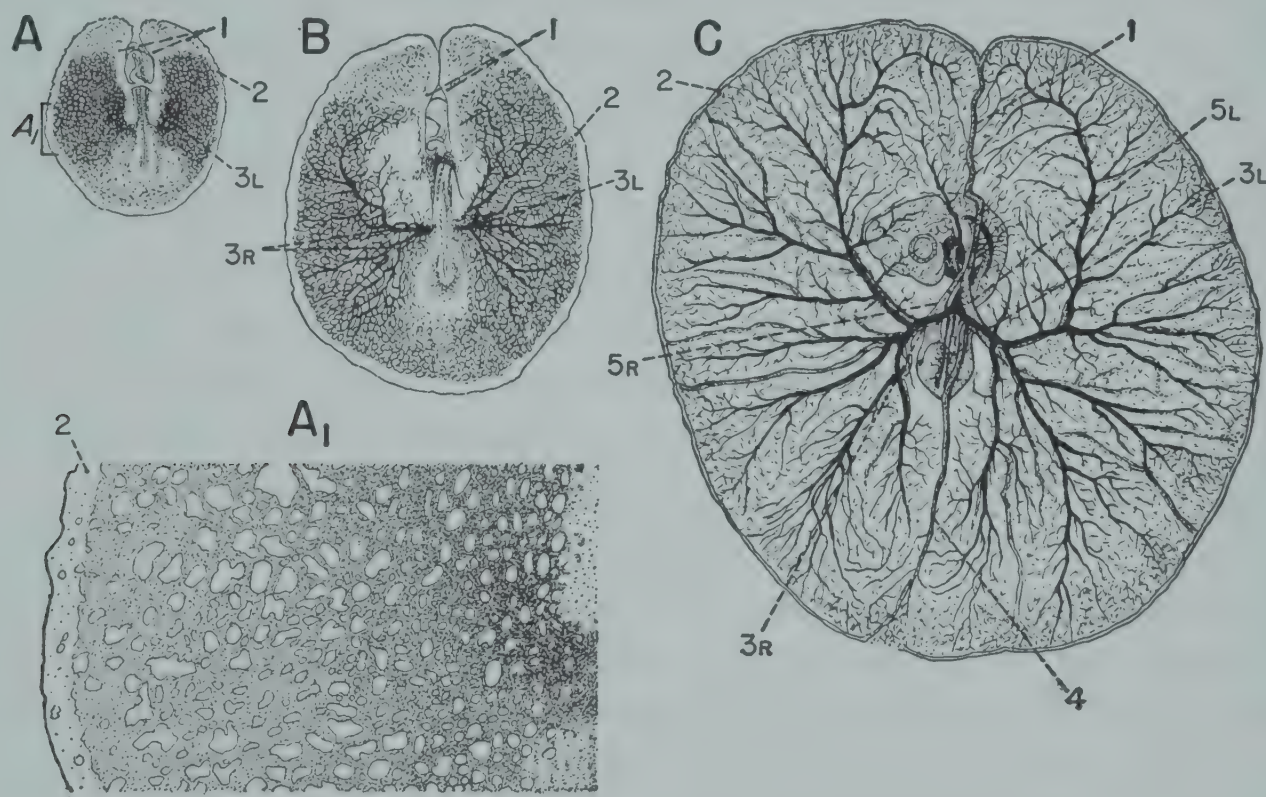
It has been suggested that the hydrolysis of yolk lecithins forms quaternary ammonium compounds which change the state of the protoplasm in the vitelline epithelial cells and cause their transformation into histiocytes (*Thomas, 1936*). This theory is based on the observation that the produc-



tion of histiocytes occurs spontaneously in only 3 per cent of cultures but can be provoked in a significantly higher proportion (up to 59 per cent) by the presence in the culture medium of quaternary ammonium compounds of the choline group (Thomas, 1936, 1937).

### The Yolk Sac Mesoderm

It will be recalled that the mesoderm of the yolk sac is the splanchnic mesoderm and that it is confined to the area vasculosa, which, of course, eventually spreads to the distal limits of the sac. The deeper portion of the mesoderm gives rise to blood vessels, and the more superficial portion becomes the connective tissue which surrounds the yolk sac externally and forms the axial trabeculae of the internal folds.



**Fig. 390.** Development of vitelline circulatory system in chick embryo, ventral view. Venous vessels are shown lighter than arterial vessels. (Redrawn with modifications after Popoff, 1894.)

A, B, and C, embryos of 48, 61, and 116 hours, respectively, with their entire vitelline circulatory systems ( $\times 2.5$ ). A<sub>1</sub>, a portion of the vitelline circulatory system of the 48-hour embryo ( $\times 12$ ).

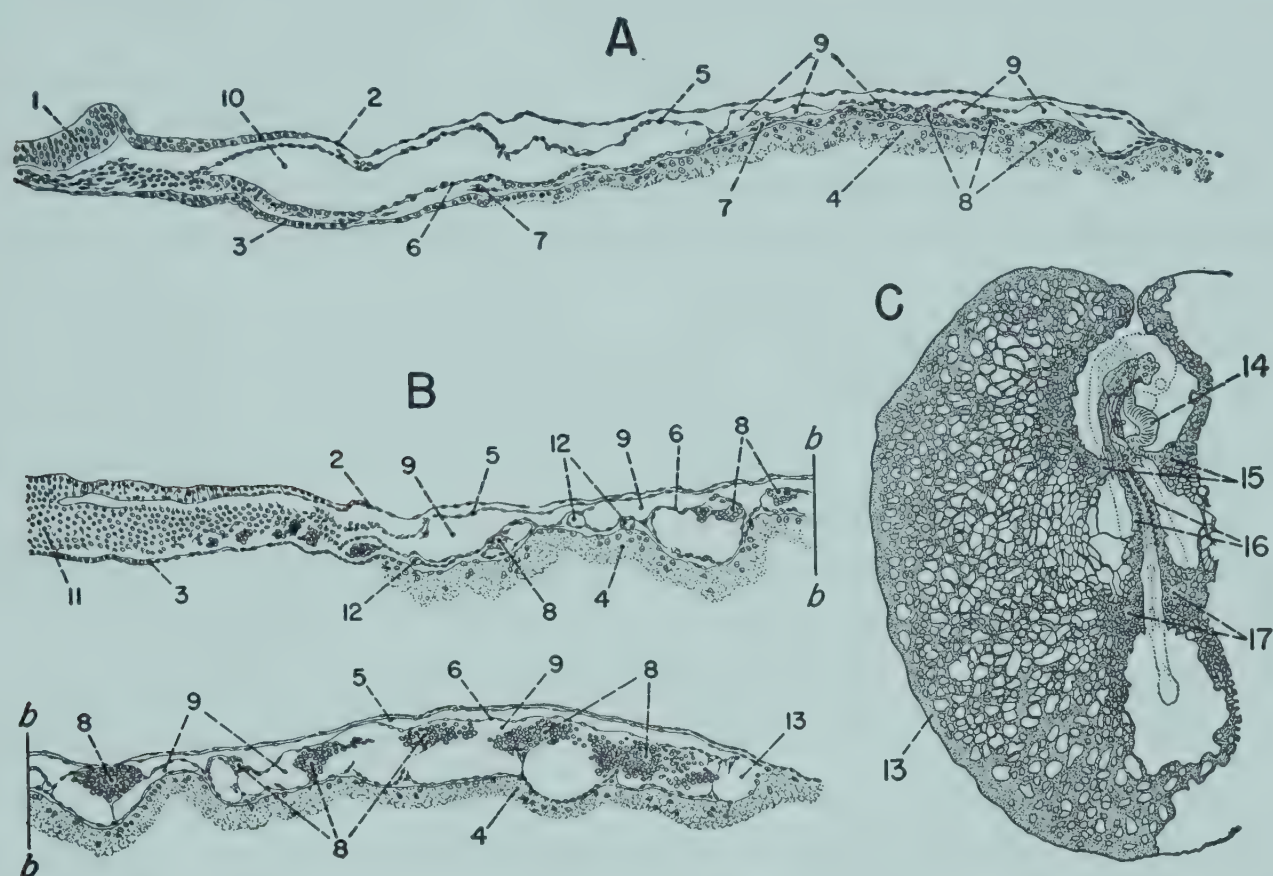
1, anterior vitelline vein; 2, sinus terminalis; 3R, right omphalomesenteric artery; 3L, left omphalomesenteric artery; 4, posterior vitelline vein; 5R, right omphalomesenteric vein; 5L, left omphalomesenteric vein.

### The Blood Vessels

The first stage in the development of the blood vessels of the yolk sac is the establishment, by self-differentiation, of a capillary network; from this network, arteries and veins are molded under the hydrodynamic influence of the circulation. At first, the blood is channeled through a simple circuit; then, as circulatory forces continue to operate, new venous vessels arise and the route of the blood is greatly altered. As a result, the develop-



ment of the vascular system of the yolk sac can be divided into several stages: first, there is the precirculatory stage in which the indifferent capillary network appears; this is followed by the stage of the primary circulation, which passes by gradual transition into the stage of the definitive circulation. Some of the stages in the development of the vascular system of the yolk sac may be seen in Fig. 390.



**Fig. 391.** Early stages in the differentiation of blood vessels in the area vasculosa of the chick's yolk sac. (Redrawn with modifications A, B, after Rückert and Mollier, 1906; C, after Chapman, 1918.)

A, cross section of the area vasculosa at the 6-somite stage, taken at a level anterior to the somites ( $\times 60$ ); B, cross section of the area vasculosa at the 12-somite stage, taken at the level of the caudal part of the body ( $\times 60$ ); C, surface view of the embryo and an area vasculosa at the 16-somite stage, showing the left half of the vascular system, as demonstrated by injection ( $\times 10$ ).

1, neural plate; 2, ectoderm; 3, endoderm of area pellucida; 4, yolk sac endoderm; 5, somatic mesoderm; 6, splanchnic mesoderm; 7, anlage of empty endothelial vesicle; 8, blood island; 9, extraembryonic coelom; 10, pericardial cavity; 11, primitive streak; 12, empty endothelial vesicle; 13, sinus terminalis; 14, heart; 15, vitelline vein; 16, dorsal aortae; 17, capillary network preceding the formation of the vitelline artery.

**The Indifferent Capillary Network.** The capillary network of the yolk sac develops in the thickened splanchnic mesoderm of the area opaca and the area pellucida. The first step in capillary formation is the differentiation of hollow vesicles from solid clumps and plexuses of mesodermal cells (known variously as blood islands, angioblasts, and hemangioblasts) which may be very massive or composed of only two or three layers of cells. The external cells of these aggregates form endothelial elements, beginning on the surface adjacent to the endoderm (Fig. 391-A). The internal cells liquefy, simultaneously forming plasma and the lumina of the vesicles (Fig.



391-B), although many of the internal cells of the larger clumps persist as blood cells, particularly in the area opaca (see Chapter 8).

The first vascular spaces arise in this manner in the distal part of the extraembryonic mesoderm. They usually appear at the 5- to 7-somite stage. Almost simultaneously, similar spaces are formed in the anterior part of the area pellucida, and very shortly afterward in the medial portion of the area opaca (*Sabin, 1920*). In general, it may be said that the differentiation of vessel primordia initially proceeds from anterior to posterior and from the area opaca to the area pellucida (*Rückert and Mollier, 1906; Hughes, 1935*). The process of blood vessel formation is described in greater detail in Chapter 8. In its early stages, it is accompanied by the development of the exocoelom, which likewise arises in the form of isolated spaces. These are dorsal to and alternate with the vascular spaces (*Rückert and Mollier, 1906; Weber, 1907a; Sabin, 1920; Jolly, 1939-40*), as Fig. 391-A shows. The initial discontinuity of the exocoelom is apparently responsible for Budge's (1887) conclusion that the yolk sac contains a system of lymphatic vessels, for Kutsuna (1933), who easily corroborated the presence of lymphatics in the allantois, was unable to find them in the yolk sac.

A continuous network of tubular vessels very quickly arises as the vascular spaces coalesce and send out sprouts, or proliferations of endothelium, which join with adjacent sprouts and develop lumina communicating with the pre-existing lumina of the parent endothelial spaces. (*Klein, 1871; Dubuisson, 1906; Sabin, 1920; Hughes, 1935*). By the middle of the chick's second day of incubation (approximately at the 16-somite stage), the vascular system of the yolk sac is sufficiently differentiated (Fig. 391-C) to permit the circulation of blood. The capillaries are interconnected and in full communication with each other except in a small region around the posterior extremity of the body, where undifferentiated blood islands still exist.

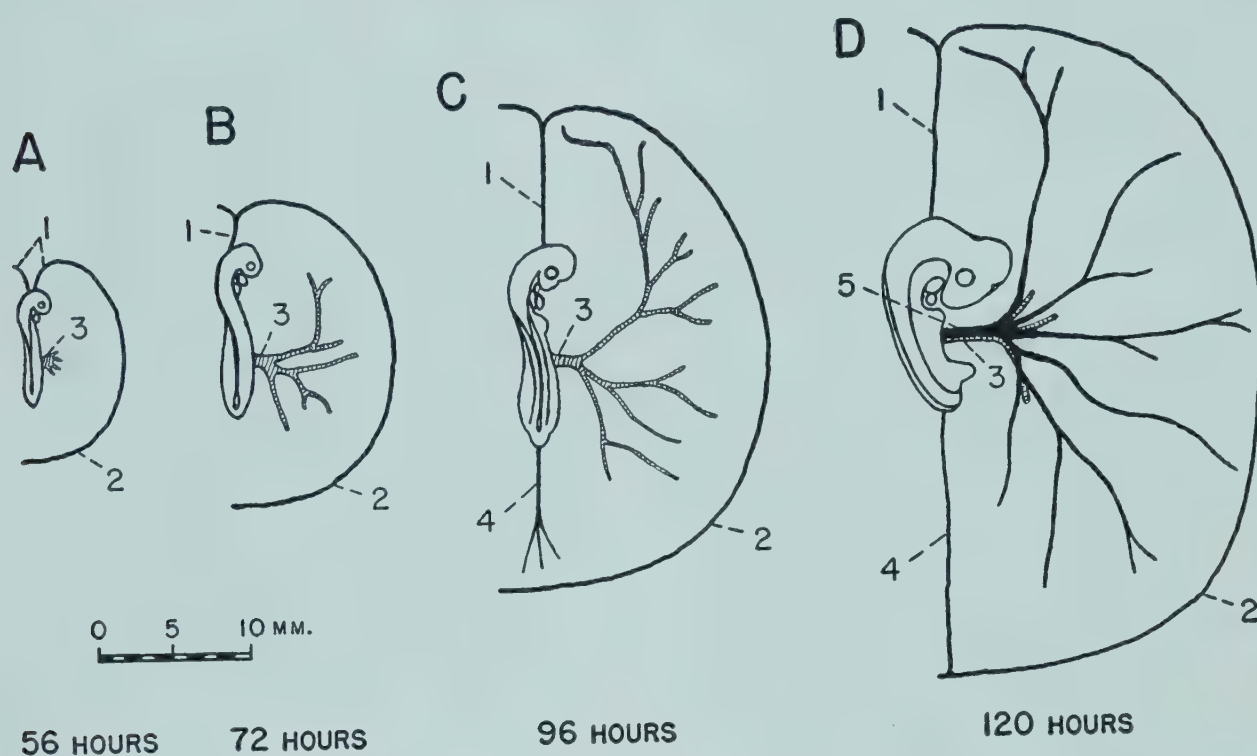
The vascular network, however, is not entirely uniform. Because of variations in the diameter of the capillary lumina and of the intervascular spaces, it is possible to distinguish three general zones (cf. Fig. 391-C), as follows: first, an inner zone of large capillaries and small interspaces, which radiates on each side from the level of the last somites; second, a middle zone of wide interspaces and narrow capillaries, radiating on each side from the level of the anterior intestinal portal; and third, a narrow peripheral zone where the vascular spaces are especially large (*Klein, 1871; Popoff, 1894; Chapman, 1918; Hughes, 1935*). The peripheral vascular spaces are already coalescing at this time to form the marginal vein or sinus (vena or sinus terminalis), which marks the outer boundary of the area vasculosa. In addition, it can be seen that the capillaries immediately adjacent to the trunk of the embryo have elongated and grown narrow with the longitudinal extension of the blastoderm opposite the somites. Mitoses, which are fre-



quent during the establishment of the capillary network, now become very rare (*Hughes, 1935*).

Development of the capillary network may be partially or entirely prevented if the incubated egg is subjected to severe jarring. Vessels do not form because the blood islands and vascular spaces fail to coalesce (*Stiles and Watterson, 1937*).

**The Vessels of the Primary Circulation.** When circulation begins, the blood naturally takes the course of least resistance through the widest capillaries. It is therefore led away from the body, since the capillaries radiating outward are larger than those paralleling the trunk of the embryo. It eventually enters the sinus terminalis and returns to the heart by way of



**Fig. 392.** Diagrams showing the development of the main vitelline blood vessels of the chick at different times during the incubation period. (Redrawn with modifications after Grodzinski, 1934b.)

A, B, C, and D, at 56, 72, 96, and 120 hours, respectively. All in scale.

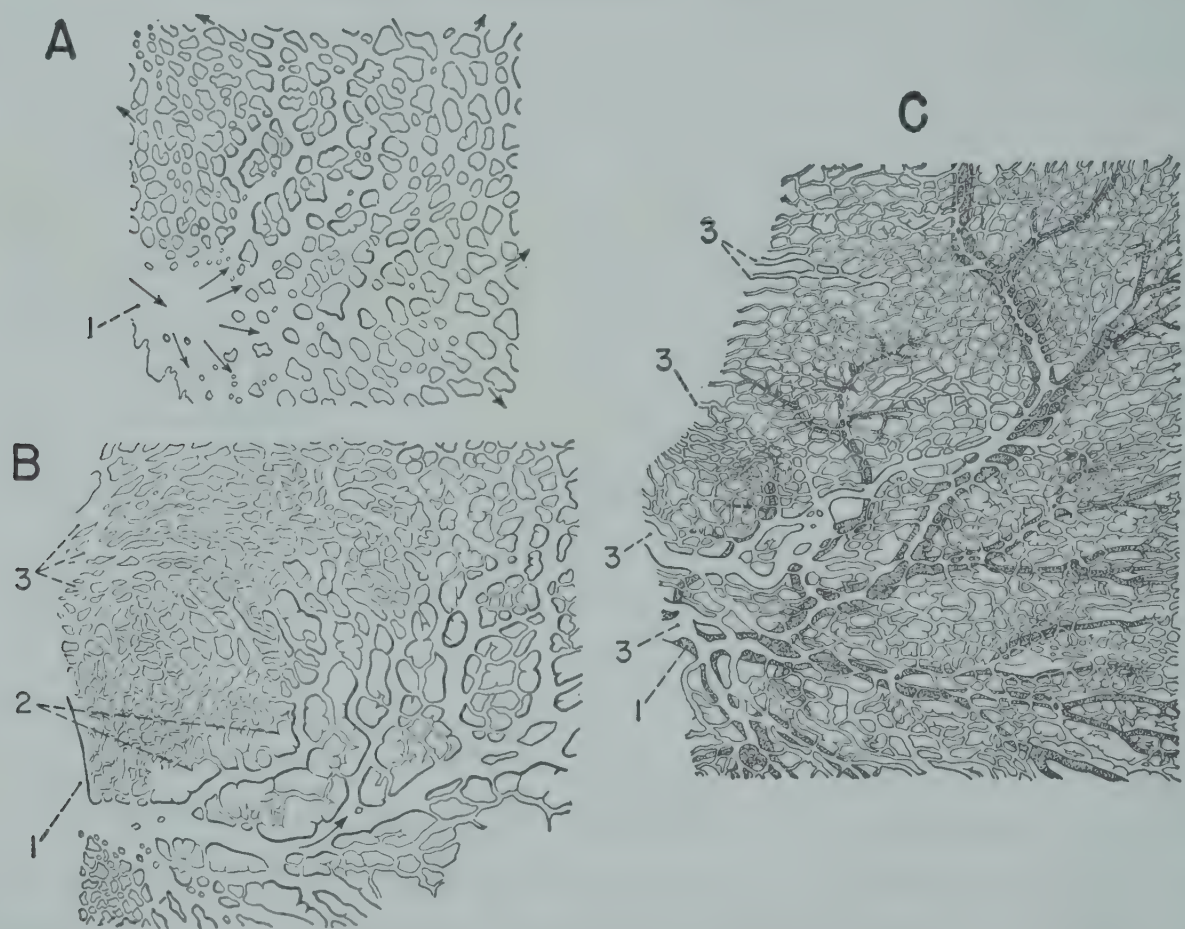
1, anterior vitelline vein; 2, sinus terminalis; 3, vitelline (omphalomesenteric) artery; 4, posterior vitelline vein; 5, lateral vitelline (omphalomesenteric) vein.

the large peripheral vascular spaces. Anteriorly, these follow the margin of the area vasculosa caudad on either side of the proamnion and lead directly to the sinus venosus. It is apparent, therefore, that the initial route taken by the blood is determined by the size of the capillaries; that is, it is determined before circulation begins, during the stage of self-differentiation (*Thoma, 1893; Hughes, 1935*). Very shortly, however, the mechanical forces of the blood stream begin to exert their influence, and other channels are formed.

By the end of the chick's second incubation day, when the blood has been circulating 12 or 14 hours, the vitelline (omphalomesenteric) arteries are clearly differentiating out of the inner radiating zone of wide capillaries (Fig. 392), where blood flow is most rapid. Most of the small inter-



vascular spaces in the path of the main blood stream have grown small and have disappeared. Simultaneously, the intervascular spaces bordering each artery have enlarged, thus isolating the arterial channel (Fig. 393-A). Some of the small capillaries between the bordering intervascular spaces have lost their connections with the artery (Fig. 393-B) by the formation of septa across the openings from the artery; the lumina of other capillaries have contracted and become obliterated (*Hughes, 1935*). The fact that the vitelline arteries do not develop after removal of the heart (although the area vasculosa continues to grow) is evidence of the importance of the



**Fig. 393.** Diagrams showing the differentiation of the chick's artery from the capillary network of the area vasculosa and the formation of a venous network dorsal to the artery as a result of the breaking of capillary connections. (Redrawn with modifications after Thoma, 1893.)

A, 48 hours ( $\times 12$ ); B, 57 hours ( $\times 10$ ); C, 74 hours; all dorsal views ( $\times 8.5$ ).

1, vitelline artery; 2, broken capillary connections; 3, venous channels or vessels.

circulation in molding these vessels out of the capillary net (*Chapman, 1918*). It is also pertinent to note that destruction of one side of the blastoderm in precirculation stages results in compensatory development of the branches of the surviving vitelline artery (*Wolff and Stephen, 1948b*).

The vitelline arteries now become circular, rather than flat, and increase in diameter. Their growth is clearly the result of the great velocity of the blood coursing through them, for, as Thoma (1893) indicated, the width of a blood vessel is determined by the rate of blood flow within the vessel. Expansion of the lumen of a vessel is equivalent, of course, to an increase in the surface area of the wall. Thoma (1893) believed that superior nutri-



tion was responsible for the faster growth of vessels containing large amounts of blood. Hughes (1935) suggested, however, that the underlying mechanism is the generation of tension in the wall of the vessel by the fluid friction of the circulation; according to his reasoning, the tension in turn causes an elongation of nuclei and of mitotic spindles in such a way that growth occurs in the direction offering the least resistance to the blood and thus relieves the tension. This hypothesis grew out of the observation that many nuclei and spindles are oriented perpendicular to the long axes of the arteries when, at the end of the chick's second incubation day, mitoses again become numerous and the vitelline arteries start to enlarge.

The differentiation of the vitelline arteries is accompanied by the final differentiation of the marginal vein from the discontinuous peripheral vascular spaces. The sinus terminalis is now a continuous vessel of wide caliber. Within the anterior "horns" of the area vasculosa and along the borders of the proamnion, the vascular spaces coalesce relatively slowly. The still distinct venous capillaries and vascular spaces of this region are the primordia of the anterior vitelline veins, which will connect the sinus terminalis and the heart. The sinus terminalis and the anterior vitelline veins apparently form by self-differentiation, for Chapman (1918) found that these vessels appear in blastoderms in which circulation is prevented by early extirpation of the heart.

During the period between the 22- and 28-somite (approximately 44- to 49-hour) stages, the marginal vein increases considerably in circumference (Hughes, 1935), without sending out sprouts (Grodzinski, 1934b). Simultaneously, the entire vascular network expands, so that the individual capillary meshes become elongated and radially arranged (Hughes, 1935). This early expansion appears to occur largely under the influence of circulatory mechanics, since mitosis is at a minimum at this time (Hughes, 1935). However, the observation that slow growth of the sinus terminalis and the capillary network may continue for a time in the absence of the circulation (Patterson, 1909a; Chapman, 1918; Remotti, 1933b; Grodzinski, 1934a, 1934b) is evidence that a certain amount of enlargement may occur by self-differentiation. The eventual regression of the vessels in blastoderms lacking a beating heart (Chapman, 1918; Remotti, 1933b) indicates that blood circulation is necessary to maintain the capillaries in tubular form (Thoma, 1893; Hughes, 1935).

By the middle of the third day, the vitelline arteries and their largest anterior, posterior, and lateral branches are clearly visible (Fig. 392-B). The blood islands posterior to the embryo have disappeared, but there is still a small vessel-free area around the tail. The marginal sinus is a well-defined vessel (Popoff, 1894).

The anterior vitelline veins, now plainly differentiated, are almost in apposition to each other in their most anterior portion (cf. Fig. 392) so



that the vessel-free region of the blastoderm (the proamnion) is out of sight beneath the embryo's head (*Popoff, 1894*). Anastomoses between the apposed parts of the anterior vitelline veins are sometimes seen at this time (*Grodzinski, 1935*), but usually they first appear after 65 to 70 hours of incubation (*Grodzinski, 1935*). As soon as connections are established between the two veins, one begins to widen and the other to grow narrower with the result that the narrower vessel eventually atrophies and disappears completely (Fig. 394). According to *Popoff (1894)*, it is the left vein that persists, but *Balfour (1873c)* and *Grodzinski (1935)* found that the right vein survives almost as often as the left, or that the definitive vessel is composed of one vein distally and the other proximally. Which vein is

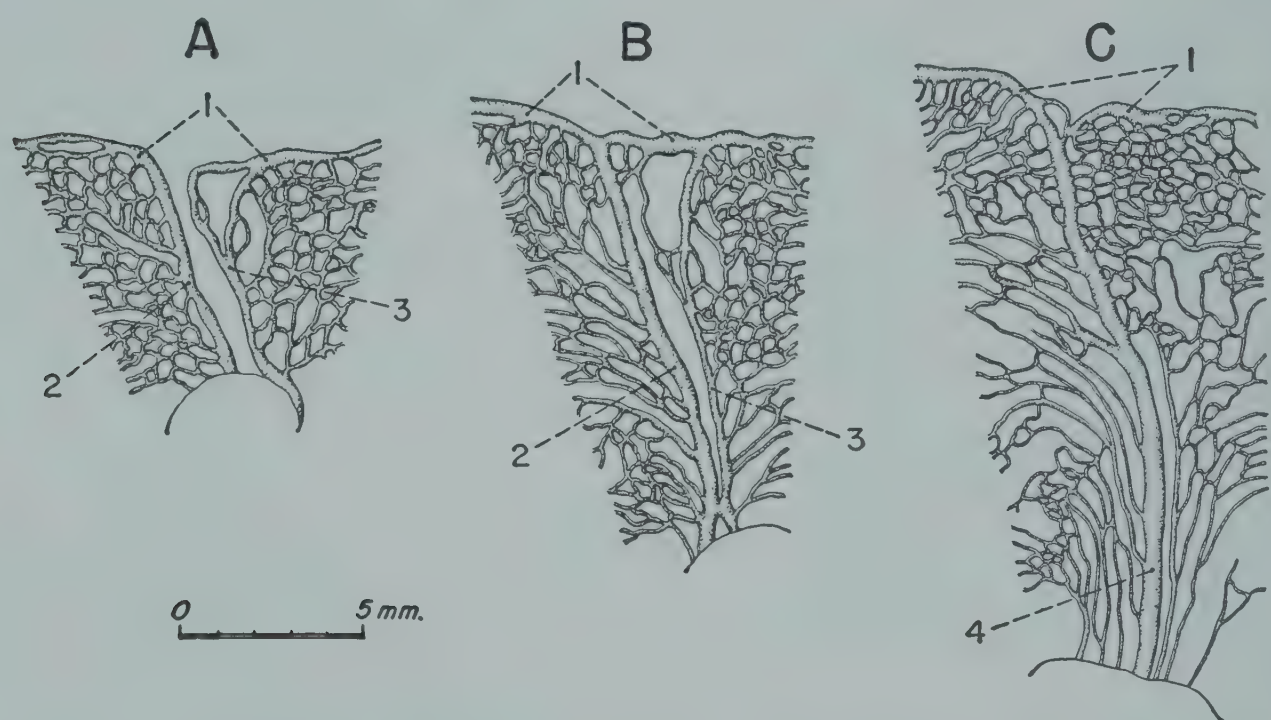


Fig. 394. Three stages in the development of a single anterior vitelline vein in the chick's yolk sac through the formation of anastomoses between the right and left vitelline veins and the subsequent atrophy of the right vein. (Redrawn with modifications after *Grodzinski, 1935*.)

A, 60 hours; B, 68 hours; C, 80 hours. All in scale.

1, sinus terminalis; 2, left anterior vitelline vein; 3, right anterior vitelline vein; 4, definitive anterior vitelline vein.

obliterated proximally may depend upon minor variations in the position of the embryo's head, whereby the right or the left vein may be compressed more than the other and thus accommodates less blood (*Grodzinski, 1935*). The increase or decrease in the diameter of the anterior vitelline veins parallels the velocity of the contained blood (*Hughes, 1937*).

The establishment of a single vessel from the paired anterior vitelline veins requires an extremely variable time (*Popoff, 1894*)—11 to 24 hours (*Grodzinski, 1935*)—but is completed before the end of the fourth incubation day, at the latest. The growth of the permanent, single vein is so rapid that *Hughes (1937)*, being unable to account for it on the basis of mitotic activity, suggested that the veins of the area vasculosa may increase



not only by cell division but also by the entry of surrounding mesenchymal cells into their endothelium.

**The Development of the Definitive Circulation.** The secondary circulation of the yolk sac differs from the primary in that the blood is returned to the heart more directly and more rapidly through newly differentiated veins that gradually supersede those of the primary circulation. The new veins arise from originally arterial capillaries which are transformed into venous vessels. The secondary circulation begins to emerge before the primary circulation is fully established, and remnants of the primary circulation persist almost to the end of incubation. Hence the two types of circulation are coexistent for the major part of the developmental period, one becoming more important while the other declining in significance.

The first venous vessels of the secondary circulation to begin their differentiation are the collateral veins, which are merely the medial portions of the lateral vitelline (omphalomesenteric) veins. They course parallel and superficial to the vitelline arteries. Their formation starts at the end of the chick's second day of incubation, when, as we have seen, medially located capillaries lose their connections with the main arterial stems. During the third and fourth days, the breaking of capillary connections progresses centrifugally, accompanying the isolation of the vitelline arteries, and is associated with the sinking of the arteries downward toward the yolk. As the arteries drop to a lower plane, the small capillaries leading directly from them bend ventralward and are obliterated (*Grodzinski, 1935*). The remaining capillaries of the network are thus brought close together, and anastomoses appear between them in the original plane (Fig. 393-C), which is now dorsal to the arteries (*Thoma, 1893; Popoff, 1894; Grodzinski, 1935; Hughes, 1935*). Many newly formed vascular spaces are incorporated into the anastomoses, hence the venous capillary network is partially self-differentiating (*Hughes, 1935*). Only the terminal arterial capillaries remain in communication with the dorsal venous plexus (*Thoma, 1893; Grodzinski, 1935*). The flow of blood through the dorsal capillaries ceases as the connecting links are severed and is resumed in the opposite direction when the anastomoses appear (*Grodzinski, 1935*). The differentiation of the collateral veins requires from 12 to 30 hours and is most rapid closest to the embryo (*Grodzinski, 1935*).

During the third day, many peripheral arterial capillaries are transformed into venous channels (*Popoff, 1894*); but the change occurs centripetally, from the sinus terminalis inward, and the blood in the capillaries continues to flow centrifugally, into the sinus (*Grodzinski, 1935*). The radially arranged veins that develop from these venous capillaries were termed primary intermediate veins by Popoff (1894), who believed that they eventually joined the collateral veins and thus became the peripheral branches of the lateral vitelline veins. According to Grodzinski (1935),



however, the collateral vitelline veins merely continue to differentiate centrifugally during the fourth and fifth days, sending branches (termed secondary intermediate veins by Popoff) between the branches of the vitelline arteries. Since the blood flows in opposite directions in the primary and secondary intermediate veins, these vessels cannot become connected. Instead, there is stasis of the blood in the intervening zone, followed by atrophy of the primary intermediate veins and their eventual disappearance and replacement by the secondary intermediate veins. The primary intermediate veins, therefore, form a part of the primary circulation.

During the third incubation day, the differentiation of the posterior vitelline vein also begins. This vein, like the anterior vitelline vein, connects the sinus terminalis and the left lateral vitelline vein; it approximately bisects the posterior half of the area vasculosa. It arises from capillaries which contain blood from the beginning and to which more is brought as soon as circulation begins. Blood from right and left flows back through the sinus terminalis to the posterior midline, where the two streams meet and are forced to find an outlet through the capillaries between the sinus and the embryo's tail. The capillaries thus become distended with blood, which remains at a standstill until the latter part of the third incubation day. At this time, the lateral vitelline veins are differentiating medially, and the blood in the posterior median capillaries now finds an outlet into the left lateral vitelline vein. During the fourth day, the single posterior vitelline vein differentiates centrifugally (Fig. 395; cf. Fig. 392) from these capillaries in much the same way as the vitelline arteries; that is, the capillaries through which the blood flows most rapidly become the largest and give rise to the definitive vessel (Grodzinski, 1935; Hughes, 1937). Its course is very variable, sometimes parallel to the embryonic axis, sometimes at an angle with it (Grodzinski, 1935). Without the influence of the circulation, the posterior vitelline vein does not appear (Chapman, 1918).

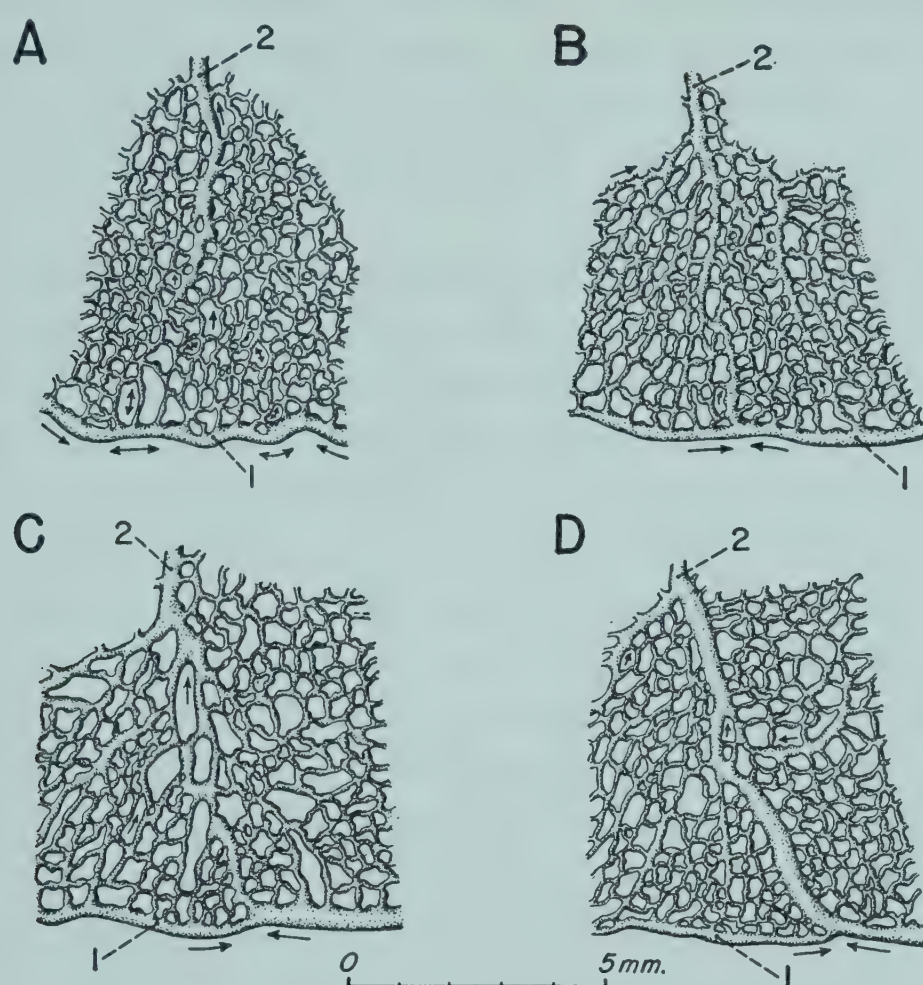
By the end of the chick's fifth incubation day, therefore, the definitive veins have differentiated (cf. Fig. 392) and the entire network of originally arterial capillaries has become venous (except in a narrow peripheral zone). This venous network is very dense, consisting of extremely fine vessels and small interspaces. The arteries retain no traces of their primitive character except in the persisting peripheral arterial capillary network. By means of numerous new branches, they have extended themselves peripherally and have formed new connections with veins proximally.

It is significant that the emergence of the definitive system of veins is accompanied by the regression of the sinus terminalis. The caliber of the sinus begins to decrease during the chick's third incubation day, when the lateral and posterior vitelline veins start to differentiate. At the stage of 40 somites, the sinus begins to break down into capillaries at the point where it is joined by the primary intermediate veins (Popoff, 1894). This



disintegration continues and is virtually completed at some time between the sixth day (*Chapman, 1918*) and the tenth day (*Popoff, 1894*), although certain portions of the sinus remain longer than others.

Within the period between the tenth and the fifteenth day there is continued formation of arterial and venous branches and the final disappearance of all but a small remnant of the arterial capillary network. By this time the arteries are deeply embedded in the tortuous folds projecting into the yolk from the internal surface of the yolk sac. Peripherally, the veins bend around to run parallel to the margin of the yolk sac.



**Fig. 395** Four stages in the differentiation of the terminal part of the chick's posterior vitelline vein from the indifferent capillary network of the area vasculosa. (Redrawn with modifications after *Grodzinski, 1935*.)

A, 70 hours; B, 77 hours; C, 82 hours; D, 91 hours. The arrows indicate the direction of blood flow.

1, sinus terminalis; 2, posterior vitelline vein. All in scale.

The development of the vitelline vessels continues up to the end of incubation. In later stages, the folds on the inner surface of the yolk sac are so large that it is possible to speak of the vessels embedded in these folds as distinct from those of the yolk sac wall. The large veins belong in the latter category. On the chick's twentieth incubation day, the anterior and posterior veins are beginning to atrophy in places, while persisting at the points where they give off branches. The lateral veins are more luxuriantly branched than ever, especially in the equatorial region. There are almost no arterics in the wall; they are confined almost exclusively to the folds, which,



as remarked before, are most highly developed equatorially and gradually disappear toward the distal pole of the yolk sac. In each fold, the arteries are completely surrounded by a network of minute venous capillaries, including two longitudinal anastomoses.

A vascular ring, composed of several branching and anastomosing vessels of varying size and inconstant arrangement, is found at the distal pole of the yolk sac. On or after the chick's sixteenth day of incubation, these vessels form connections with the vascular system of the allantois (*Popoff, 1894; Fülleborn, 1895; Spampani, 1905; Steinmetz, 1930*).

**Histological Development of Yolk Sac Vessels.** During the course of incubation, the walls of the blood vessels in the area vasculosa undergo a certain amount of histological development, which is most marked in the large arteries near the embryo. The structural changes were described by Cohn and Lange (1930).

In the beginning, the walls of arteries and veins alike are composed of a single layer of endothelial cells. By the chick's tenth day of incubation, the veins near the embryo are irregular in shape and are surrounded by an additional layer of large, loosely arranged cells. By contrast, the arteries in this region are round, and their walls consist of two to five layers of circularly arranged cells. In the largest arteries, the cells of the innermost layer are somewhat taller than the rest; there are a few delicate collagen fibers between the cells, but no elastic fibers. Remote from the embryo, the arteries retain their primitive structure.

At the 14-day stage, the veins close to the embryo are of larger diameter than the arteries and consist of one layer of endothelium surrounded by two outer layers of very loosely meshed cells. The largest adjacent arteries are composed of an endothelial lining one cell thick, surrounded by a layer of longitudinal muscle three cells thick, a layer of circular muscle seven cells thick, and an irregular outermost layer of loosely arranged connective tissue (adventitial) cells. Collagen fibers are present within the muscle and connective tissue layers, but elastic fibers are still absent. Somewhat farther from the embryo, the larger arteries are two to five cell layers thick, but the arteries at the greatest distance can be distinguished from capillaries only by their size. Dubuisson (1906) noted that the yolk sac of the 15-day embryo is delimited externally by three layers of connective tissue cells, and that the blood vessels are found in the innermost layer where the connective tissue penetrates into the folds on the internal surface.

After 18 days' incubation, the arterial walls near the embryo are thicker than previously but otherwise unchanged. Some of the most distally located arteries now have walls two to five layers thick, but the smallest still resemble capillaries structurally. Elastic fibers are present only at the point of transition to the embryo, in a region which must be regarded as embryonic rather than extraembryonic.



On the hatching date, there are no indications of degeneration of the yolk sac blood vessels. Anatomically, the arteries of various calibers are the same at this time as during development.

Staining reactions have failed to reveal the presence of any vasomotor nerves in the area vasculosa of the chick (*Cohn, Lange, and Ehrich, 1930*) from the third to the twentieth day of incubation (*Lange, Ehrich, and Cohn, 1930.*)

**The Irritability of Yolk Sac Vessels.** The reactions of the noninnervated blood vessels of the chick's yolk sac to certain stimuli have been investigated mainly for comparison with the reactions of innervated blood vessels. Although yolk sac vessels, like innervated vessels, vary systematically in their response according to the strength of the stimulus, their reactions differ in some important respects from those of vessels possessing vasomotor nerves.

Electrical, mechanical, and various chemical stimuli (ammonia, silver nitrate, potassium iodide, oil of camphor, and mustard oil) are effective (*Cohn, Lange, and Ehrich, 1930; Lange, 1930a; Lange, Ehrich, and Cohn, 1930; Volterra, 1935*). Regardless of its nature, a weak stimulus elicits dilation of yolk sac capillaries, a stimulus of medium strength has the opposite effect, and a strong stimulus results in stasis of the blood in a localized dilatation occurring between two contracted segments. The arterial subdivisions differ in irritability, for, at any incubation age, progressively stronger stimuli are required to bring forth the above responses in small arterial branches and in main arterial stems (*Cohn, Lange, and Ehrich, 1930; Lange, 1930a*). The gradations of irritability are directly related to the thickness of the arterial walls. Another histological feature of yolk sac arteries, namely, the absence of elastic fibers in their walls, apparently accounts for the fact that they contract by flattening rather than by constriction (*Cohn and Lange, 1930*).

The effect of a stimulus is confined to the immediate region in the area vasculosa which is stimulated, and does not spread to the surrounding vessels. In their restricted reaction, yolk sac vessels differ from innervated vessels (*Cohn, Lange, and Ehrich, 1930; Lange, 1930a; Lange, Ehrich, and Cohn, 1930*). The absence of nerve conduction in the area vasculosa is shown by the fact that the entire vascular region can be isolated from the embryo by a circular incision, yet the response of its vessels remains unchanged (*Lange, Ehrich, and Cohn, 1930*).

It has been said that yolk sac vessels also differ from innervated vessels in that they do not respond to adrenaline (*Cohn, Lange, and Ehrich, 1930; Lange, Ehrich, and Cohn, 1930*) or to atropine (*Lange, 1930a*). This statement was questioned by Volterra (1935), who found that adrenaline, in direct contact with the walls of small vessels, always causes contraction after a short latent period, and that atropine reinforced the effect of



adrenaline. The same author also reported that acetylcholine acts as a vasodilator, although a prior application of atropine may inhibit it. Ergotamine was found to produce constriction. Heat and cold may have no distinct effect (*Lange, 1930a*), or may cause dilatation and contraction, respectively (*Volterra, 1935*).

Experiments performed at the 4-day stage have shown that the yolk sac blood vessels do not react to acid ( $pH$  5) or alkali ( $pH$  9) but respond to carbon dioxide or the carbonate ion by contracting. It was suggested that sensitivity to carbon dioxide is a mechanism whereby blood flow is regulated at different temperatures (*Hammett and Zoll, 1928*), since metabolic rate and carbon dioxide production presumably change with the temperature. In a concentration too small to affect the diameter of the yolk sac vessels, carbon dioxide appears to lower their threshold of irritability to electrical stimuli (*Lange, 1930b*).

### *The Connective Tissue*

The smooth outer wall of the yolk sac consists of connective tissue derived from the single layer of flattened splanchnic mesodermal cells that originally comprises the floor of the exocoelom (*Kölliker, 1884*). This layer of cells appears first between the primordia of the blood vessels and does not form directly above them until the vascular endothelium has differentiated (*Rückert and Mollier, 1906*).

During the second half of the embryonic period, the yolk sac mesoderm increases in thickness and assumes the characteristics of connective tissue. On the chick's fifteenth day of incubation, for example, it consists of at least three layers of cells and is striated tangentially with fibrils. The nuclei in the outermost layer are the largest; those in the inner layers, which contain the blood vessels, are flat and elongated. Meantime, the mesoderm has been incorporated into the folds on the inner surface of the yolk sac, as previously mentioned, and the axial trabeculae of the folds now consist of similarly thickened connective tissue continuous with the connective tissue of the yolk sac wall (*Dubuisson, 1906*).

### *Blood Formation in the Yolk Sac Mesoderm*

An extremely important function of the yolk sac is the formation of blood cells which are mesodermal derivatives. Their extraembryonic production continues until a late date and gradually dwindles after hematopoiesis is established within the embryo's body. The entire subject of blood cell manufacture is discussed in Chapter 8.

### *The Absorption of Yolk*

It was remarked by Haller (1758) that the endoderm of the yolk sac is continuous with the gut endoderm and that the yolk sac is essentially an



extension of the intestine. It is not surprising, therefore, that this membrane functions in a digestive capacity and performs the first step in the process of yolk assimilation. Despite the communication between the gut and the yolk sac, no yolk ordinarily passes into the intestine during the incubation period, or at least not until very close to its end. Instead, enzymes elaborated by the yolk sac endoderm attack the constituents of the yolk, and the products of enzymatic activity are transferred from the endodermal cells to the blood vessels of the yolk sac and thence to the embryo. The injection of various compounds (such as promin, sulfathiazole, and radioactive dibasic sodium phosphate) into the yolk has shown that this is the route of absorbed substances (*Lee, Stavitsky, and Lee, 1946; Taylor and Saenz, 1949*). At the end of incubation, the weight of the yolk sac's contents has been reduced by about 60 per cent, and the remainder of the yolk is absorbed after hatching.

The physiological activity of the yolk sac endoderm is correlated with its morphology. The ability to secrete enzymes evolves gradually with the organization of the cells into an epithelium. During the yolk sac's formative period, spheres of yolk are apparently engulfed by the endodermal cells of the area vitellina (*Virchow, 1891; Dubuisson, 1906; Remotti, 1927b; Kono-packa, 1933*). Although the phagocytosis of yolk has never actually been observed, it has been assumed to occur because the intracellular and extracellular yolk spheres are at first identical in appearance and in fluorescent properties (*Zanoni, 1933*); furthermore, cultured endodermal cells from the area vitellina have been seen to phagocytize India ink granules (*Grodzinski, 1930; Thomas, 1938*). Schaper (1902), who injected a suspension of carmine powder into the yolk of the 6-day incubated chicken egg and found intracellular carmine particles in all zones of the yolk sac endoderm 4 days later, concluded that the organized epithelial cells are phagocytes no less than the more distal elements. "Ultraphagocytosis," involving deformation of the superficial cytoplasm, is perhaps indicated (*Thomas, 1938*) by the ability of endodermal cells from all regions of the yolk sac to absorb colloidal dyes injected into the yolk *in vivo* or added to the culture medium *in vitro* (*Wislocki, 1921; Latta and Busby, 1929; Thomas, 1938*). In the opinion of several investigators (*Virchow, 1891; Dubuisson, 1906; Remotti, 1927b*), the organized epithelial cells have the capacity for phagocytosis only during the early stages and then gradually lose it, although it is possible that they may regain it in the presence of a foreign substance (*Remotti, 1927b*). Dubuisson (1906), noting a regular alternation between empty and engorged epithelial cells on the first incubation day, suggested that the cells are incapable of phagocytosis while full of yolk and recover this power when they have disposed of their inclusions. Thomas (1938), on the other hand, concluded that the presence of yolk in the process of being digested is largely responsible for the phagocytic properties of cul-



tured endodermal cells from the area vitellina. He also believed that the first yolk spheres visible in the endodermal cells are not inclusions but intracellular secretions which are partially digested within the cell but are then excreted into the yolk, thus serving as vehicles for the transport of enzymes.

As the yolk-laden endodermal cells make the gradual transition to columnar epithelial elements, they mature physiologically as well as morphologically. Their functional development is indicated by the fact that their yolk inclusions show a progressive modification in appearance from the distal to the proximal part of the yolk sac. The intracellular yolk spheres in the peripheral zones give the staining reactions of both proteinous and fatty substances, whereas most of those in the epithelial zone stain like lipoids (*Konopacka, 1933*). It appears, therefore, that proteins are removed by intracellular digestion (*Dubuisson, 1906; Remotti, 1927b*). The process of yolk digestion, however, is not well understood. Thomas (*1938*) and Grodzinski (*1946*) investigated it in cultures of endoderm from the area vitellina but drew divergent conclusions. Thomas (*1938*) considered yolk to be a lipoprotein complex and believed that intracellular lipases and proteases gradually digest it and leave only globules of glycerides. He claimed that it is possible to identify, during the course of digestion, such substances as lecithin, cholesterol, cholesterol esters, fatty acids, glycogen, amino acids and their derivatives (sulfhydryl groups, phenolic and indolic compounds), iron (ferric) compounds, organic sulfur, peroxidases, and phenolases. Grodzinski (*1946*) regarded the yolk spheres as vesicles of fluid colloidal protein containing neutral fat droplets and surrounded by a semipermeable membrane of lipoids. According to his description of the intracellular digestion of yolk, lipases dissolve the membrane of the yolk sphere and release the fat droplets, which disperse in the cytoplasm of the cell while simultaneously fusing into larger droplets. The protein of the yolk sphere is incorporated into the cellular protoplasm, and the fat droplets undergo further digestion, which transforms them from isotropic to anisotropic substances, perhaps from glycerides to phosphatides.

As the organization of the yolk sac epithelium progresses, enzymes produced or activated within the cells are excreted into the yolk in increasing quantities. The close relationship between morphological and functional development is shown by the observation that proteolytic and autolytic activity is marked in cultures of yolk sac epithelium from the area vasculosa but is much less pronounced in cultures of endoderm from the area vitellina (*Thomas, 1933, 1938*). Presumably, phagocytosis and intracellular digestion are gradually replaced by the more efficient process of extracellular disaggregation of yolk followed by absorption of the soluble products of enzymatic activity (*Remotti, 1927a, 1927b*). The earlier type of digestion persists longest, of course, in the distal parts of the yolk sac, where it still



occurs on the chick's eighth or tenth day of incubation (*Remotti, 1927b*), or even later (*Remotti, 1930b*).

The early formation of extracellular enzymes by the yolk sac is indicated at the end of the chick's first day of incubation by the liquefaction of the most superficial part of the yolk as far distally as the outer boundary of the area vitellina interna (*Virchow, 1891*). At this time, liquefied yolk occupies a narrow slit, the perilecithal space, which is continuous with the subgerminal cavity (*Baer, 1828b; Virchow, 1891*). *Virchow (1891)* noted that the perilecithal space extends itself peripherally by the confluence of small, discontinuous spaces filled with a fluid, which he regarded as the product of enzymatic activity. Later, deeper layers of the yolk are also affected, and the white yolk of the latebra becomes mingled with the yellow yolk (*Grodzinski, 1946*). The progressive liquefaction of the yolk up to the eighth day of incubation—or longer, at abnormal incubation temperatures (*Romanoff, 1943a*)—may be due in part to the inflow of water derived from the albumen; but if so, the water serves as a vehicle for enzymes elaborated by the yolk sac epithelium (*Remotti, 1927b*). The fluid yolk is more finely granular and more homogeneous than the unchanged substance (*Remotti, 1927b*). By the thirteenth or fifteenth day of incubation (*Dubuisson, 1906; Orru, 1931; Romanoff, 1943a, 1943b*), the yolk's fluid fraction is no longer identifiable as a separate entity; however, if the incubation temperature is abnormal, fluid yolk may accumulate in larger amounts and persist longer (*Romanoff, 1943a*). Usually the yolk is entirely homogeneous by the fifteenth day (*Dubuisson, 1906*), except for the presence of large fat bodies and, later, of concretions composed of concentric layers with radial striations (*Courty, 1848; Duval, 1884e; Virchow, 1891; Dubuisson, 1906; Grodzinski, 1946*). These concretions have been variously identified as lecithin (*Duval, 1884e*), calcareous substances (*Dubuisson, 1906*), cholesterin (*Remotti, 1927b*), and "fat" (*Grodzinski, 1946*). This total transformation of the yolk can be attributed to the action of enzymes (*Dubuisson, 1906*).

Histochemical studies (*Konopacka, 1933*) have revealed something of the manner in which enzymes attack the yolk. On the sixth day, for example, spheres of yolk in the yolk sac cavity are disaggregated into their constituent proteins and lipids. The globules of protein are dissolved as they approach the yolk sac epithelium, and the products of their breakdown are taken up by the cells, along with the fatty substances. *Remotti (1930b)* suggested that fats are resynthesized within the cells from absorbed products of lipolysis. According to *Konopacka (1933)*, the lipids are broken down intracellularly and the products of their decomposition pass into the yolk sac vessels in a dissolved or colloidal state and again reconstruct lipids in the vitelline veins and the embryonic vessels. It is the plasma, not the blood cells, that transports these substances.



Quantitative investigation of enzymatic activity in the yolk and yolk sac (*Remotti, 1927a, 1927b, 1930b; Calzoni, 1930*) has shown that proteolysis begins to increase earlier than lipolysis and attains a maximum level sooner. Proteolytic activity in the yolk sac membrane becomes continually more intense from the chick's second or third day of incubation until the tenth day, after which it remains approximately constant. In the yolk itself, there is a simultaneous but smaller accentuation, which is more marked and begins sooner in the proximal than in the distal yolk and which roughly parallels the yolk's increasing liquefaction. Lipolytic activity rises rapidly from the fifth or sixth day of incubation until the eighth or ninth day and more gradually until the fifteenth day, and then declines very slightly. The injection of 8-day yolk into a 15-day yolk sac, or vice versa, does not modify the normal absorption of the yolk constituents. Therefore, the order in which enzymes are elaborated by the yolk sac appears to be determined not by the type of material available, but rather by factors intrinsic in the epithelial cells; and these factors are probably in turn correlated with the condition of the entire organism, and especially with its successive metabolic requirements (*Remotti, 1938*).

The earlier increase in proteolytic activity, as compared with lipolytic activity, is consistent with the results of studies on embryonic metabolism, which have shown that the predominant utilization of carbohydrates is followed by the utilization first of proteins and then of fats (see Chapter 8). The later maximum in lipolysis is also consistent with various other circumstances, such as the great abundance of fatty inclusions in the yolk sac epithelium during the latter part of the incubation period (*Dubuisson, 1906; Remotti, 1927b*) and the rapid decrease in the percentage of fat in the dry matter of the yolk after the twelfth day of incubation (*Romanoff, 1932*).

Calculations by Needham (1933) have shown that most of the material absorbed by the yolk sac during the first week of incubation is used for the growth of the membrane itself. The absorption rate (defined as the storage and combustion increment of yolk sac, allantois, and embryo as a percentage of yolk sac weight for each day) is over 300 per cent on the second day, but it drops to about 100 per cent by the fifth or sixth day and oscillates about this level for the remainder of the developmental period. Calculation of the "transit rate," or rate of passage of absorbed material to the embryo and allantois relative to yolk sac weight, showed that the yolk sac never absorbs more than its own weight of material per day for distribution to other structures.

On the day before the chick hatches, there is an abrupt acceleration in the rate at which yolk disappears (*Romanoff and Romanoff, 1933*). It is possible that yolk is forced through the yolk stalk and into the intestine



during the retraction of the yolk sac into the abdominal cavity. Yellowish or greenish material resembling the yolk sac's contents has been found in the intestine on the last 3 days of incubation (*Haller, 1758, pp. 138-159; Knake, 1935*).

The amount of yolk left unassimilated at the time of hatching is very variable but is usually between 5 and 8 gm., in the chicken egg (*Virchow, 1891; Iljin, 1917; Romanoff and Romanoff, 1933; Entenman, Lorenz, and Chaikoff, 1940; Romanoff, 1944b*). The size of the egg, of course, is a determining factor; thus about 6 gm. of yolk remain in an egg weighing 47 gm. at the start of incubation, and about 7 gm. in one originally weighing 58 gm. The residual yolk constitutes approximately 18 per cent of the newly hatched chick's total weight (*Jull and Heywang, 1930*). The ratio between the weight of the newly hatched bird and the weight of reserve yolk is not constant in different species of domesticated and semidomesticated gallinaeous and anseriform birds, for the smaller species retain relatively less yolk than the larger species. Unusually large amounts of residual yolk are found in eggs of the chick (*Romanoff, 1943a*), the ring-necked pheasant (*Phasianus colchicus*), and the bobwhite quail (*Colinus virginianus*) incubated at abnormal temperatures (*Romanoff, 1944b*).

#### *The Postembryonic Absorption of Yolk*

Almost all the residual yolk disappears during the first 5 to 7 days after the chick hatches (*Romanoff and Romanoff, 1933; Entenman, Lorenz, and Chaikoff, 1940; Romanoff, 1944b*). The rate of yolk assimilation is thus much more rapid than during the embryonic period (*Romanoff and Romanoff, 1933*). It is likely that not insignificant amounts of yolk pass through the yolk stalk into the intestine of the hatched bird and are digested there. Virchow (1891) rejected this possibility because, in attempting to trace yolk into the small intestine, he could follow it no farther than the distal end of the yolk stalk. Kar (1947), however, found the yolk stalk of the 5-day-old chick to be full of yolk, which appeared to be in the process of absorption by the epithelium; and Trotti (1933a) observed that the injection of additional yolk into the yolk sac induces dilatation of the yolk stalk lumen and the direct passage of yolk into the intestine. Of greatest weight is the experiment performed by Knake (1935). Trypan blue injected into the yolk sac of newly hatched chicks subsequently appeared in the intestinal contents and the feces. The dyed material passed through the small intestine very rapidly and was stored in the caeca for a considerable time.

The rate of yolk assimilation varies greatly in different individuals (*Romanoff, 1944b*) but is not materially affected in chickens, turkeys (*Meleagris gallopavo*), or pheasants (*Phasianus colchicus*) by the intake of



food or water (*Schilling and Bleecker, 1928; Parker, 1929; Heywang, 1940; Romanoff, 1944b*) or (in chickens) by extreme brooding temperatures (*Parker, 1929*).

In the chicken, the various constituents of the yolk are not removed at the same rate, although proteins (*Romenski, 1919*) and fatty substances (*Romanoff, 1944b*) are both utilized rapidly. During the first week after hatching, the disappearance of neutral fat is most striking, but of phospholipid somewhat less so; free cholesterol decreases in absolute but not in relative amount; and the proportion of cholesterol esters (as per cent of wet weight) rises while the actual quantity diminishes, probably because these substances are absorbed more slowly than the others (*Entenman, Lorenz, and Chaikoff, 1940*).

The residual yolk is yellowish or greenish brown in color. Microscopic examination shows that it is composed chiefly of dense aggregations of yolk mingled with larger numbers of the radially striated concretions than are present before hatching (*Virchow, 1891; Dubuisson, 1906; Remotti, 1927b*). Charbonnel-Salle and Phisalix (1886) observed the latter formations in the residual yolk of the pigeon (*Columba livia*) and suggested that they result from the removal of fatty substances.

It has been suggested that estrogens present in the residual yolk act on the parathyroid glands to produce a temporary condition of osteitis fibrosa in the chick's humerus, thus facilitating penetration of the air sac (*Bremer, 1940a*). Removal of the yolk sac, however, does not appear to prevent pneumatization of the humerus (*Landauer, 1944*).

### Postembryonic Changes in the Yolk Sac and the Yolk Stalk

After it is taken into the body of the embryo, the yolk sac membrane undergoes involution and its contents are almost completely absorbed. The yolk stalk, on the other hand, elongates and remains for a long time, or permanently, as a diverticulum of the small intestine (Meckel's diverticulum).

Age of Chick (days)	Weight of Yolk Sac (gm.)
0.5	5.34
1.5	3.34
3.0	2.5
3.0-4.0	0.6
5.0-7.0	0.43-0.05

The yolk sac regresses at an extremely variable rate. Hence it may persist in the chicken for an inconstant time, for 2 to 4 weeks (*Schilling and Bleecker, 1928; Maumus, 1902*) to as much as 34 weeks (*Latimer, 1924*), or perhaps even for life (*Remotti, 1931*). Its involution appears to be a phenomenon related not so much to the age of the chick as to the exhaus-



tion of the yolk, for, if additional yolk is injected into its cavity, the yolk sac retains its structural integrity for a considerably longer time than usual (Remotti, 1931, 1935b). Normally, the shrinkage of the yolk sac is very rapid during the first week after hatching, as the accompanying data indicate (Virchow, 1891).

The regression of the wall of the yolk sac occurs by the processes of cicatrization and phagocytosis (Virchow, 1891; Dubuisson, 1906). Accumulations of leucocytes, especially eosinophiles, can be seen around the yolk sac vessels on the day the chick hatches (Dubuisson, 1906). On the next day, the connective tissue is becoming thicker and more fibrous. It consists of an outer layer where the fibers are tangential, a middle layer where they are perpendicular to the surface, and an inner vascularized layer (Virchow, 1891; Dubuisson, 1906). The outer layer probably corresponds to the connective tissue wall of embryonic stages, and the middle layer is probably produced by the shriveling of the outer layer (Dubuisson, 1906). The axial connective tissue of the folds is still thin. The epithelial cells have decreased somewhat in height (Virchow, 1891). After the end of the fourth day, they are disposed in several layers, and the number of folds has decreased considerably. The endothelial cells lining the blood vessels detach themselves and migrate into the lumina of the vessels, perhaps by amoeboid movement. The connective tissue of the folds now begins to thicken. Some of the connective tissue cells appear to be transformed into macrophages. These absorb the fibrous tissue, leaving vacuoles in its place, and perhaps dispose of some of the epithelial cells also (Dubuisson, 1906). White blood cells, including mononuclears and eosinophiles, are found free in the connective tissue, especially at the bases of the folds; some leucocytes are applied directly against the epithelial cells or have penetrated into them (Virchow, 1891; Dubuisson, 1906). Although many of the epithelial cells are phagocytosed, others fall into the residual yolk substance and degenerate. After a time, the yolk sac wall consists entirely of connective tissue, in which a few remnants of blood vessels and epithelial cells may be found temporarily (Dubuisson, 1906). Essentially the same changes as the above have been observed in the yolk sac of the pigeon, *Columba livia* (Charbonnel-Salle and Phisalix, 1886).

Within the first few weeks after hatching, the yolk sac seems to show an increasing tendency to become detached from the yolk stalk, and, with its residual contents, it thus assumes the status of a foreign body in the abdominal cavity (Latimer, 1924; LeMasurier, Branion, and Marcellus, 1946). Observations made on the pigeon (*Columba livia*), the chaffinch (*Fringilla coelebs*), and the hedge sparrow (*Prunella modularis*) indicate that the yolk sac then adheres to the viscera and is eventually resorbed (Charbonnel-Salle and Phisalix, 1886).

During the chick's first week of postembryonic life, while the yolk sac



is shrinking, the yolk stalk grows in length from 3.0 mm. to 10.0 mm., and its wall increases from 0.75 mm. to 1.0 mm. in thickness (*Virchow, 1891*). The opening of the yolk stalk into the intestine is in the form of a papilla (*Virchow, 1891*); a little more than 40 per cent of the total length of the intestine intervenes between the anus and this papilla (*Maumus, 1902*).

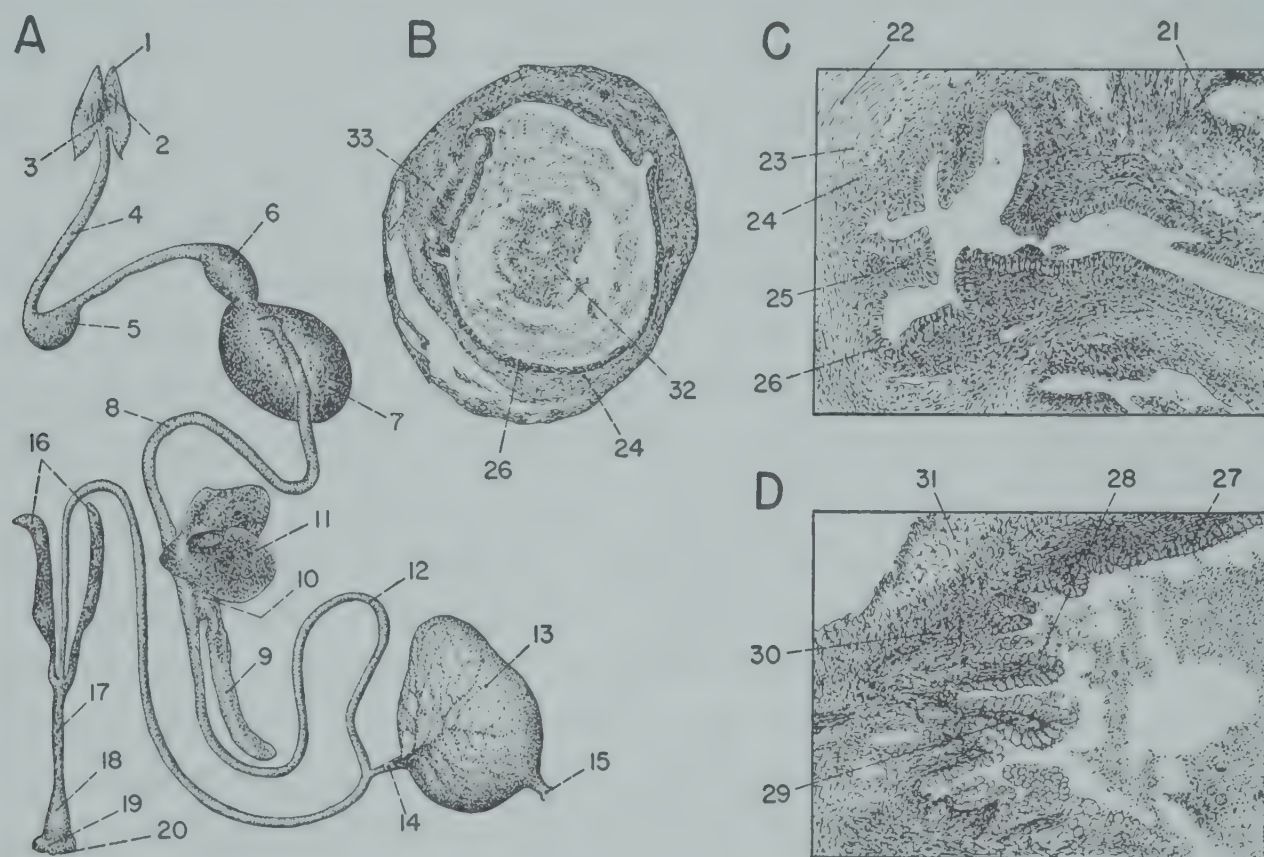


Fig. 396. The location and structure of the yolk sac and yolk stalk (as the third caecum) in the chick after hatching. (Redrawn with modifications B, after Kar, 1947; C and D, after Calhoun, 1933; A, drawn from the author's data, unpublished.)

A, the entire digestive tract of a baby chick, showing the location and appearance of the yolk stalk and the remnant of the yolk sac ( $\times 0.5$ ); B, transverse section through the yolk stalk of a 5-day-old chick, with the yolk in the lumen ( $\times 10$ ); C, section of yolk stalk from 1.5-day-old chick ( $\times 65$ ); D, section of yolk sac from 1-day-old chick ( $\times 65$ ).

1, beak; 2, tongue; 3, pharynx; 4, esophagus; 5, crop; 6, proventriculus; 7, gizzard; 8, duodenum; 9, pancreas; 10, gall bladder; 11, liver; 12, jejunum and ileum; 13, yolk sac; 14, yolk stalk; 15, navel; 16, caeca; 17, rectum; 18, cloaca; 19, bursa cloacae; 20, anus; 21, intestinal mucosa; 22, lamina muscularis; 23, submucosa; 24, muscularis mucosa; 25, tunica propria; 26, columnar epithelium; 27, cuboidal epithelium of yolk stalk; 28, columnar epithelium of yolk sac; 29, folds in the mucous membrane; 30, fibrous connective tissue layer; 31, serosa; 32, yolk; 33, connective tissue stroma.

The structure of the wall of the yolk stalk at hatching time resembles that of the intestinal wall (*Giacomini, 1893; Maumus, 1902; Lelièvre and Retterer, 1910; Calhoun, 1933; Kar, 1947*). It is composed of a serous layer, two layers of smooth muscle (an outer longitudinal and an inner circular layer), connective tissue stroma, and mucosa lined with glandular epithelium but without villi (Fig. 396). The epithelial cells are transitional between those of the intestine and those of the yolk sac (*Virchow, 1891; Trotti, 1933a*). If additional yolk is injected into the yolk sac, the lumen



of the yolk stalk widens, and its distal end dilates and becomes lined with folds resembling those on the inner surface of the yolk sac (*Trotti, 1933a*).

In the duck (*Anas platyrhynchos*), the distal part of the yolk stalk atrophies, but the proximal part persists and grows to a length of 1.7 cm. during the first 6 months of life. The epithelial glands are transformed into follicles (*Lelièvre and Retterer, 1910*). In chickens 4 months old, the stump of the yolk stalk is present and about 5.0 mm. long, and its free end is occluded (*Kar, 1947*). As to the ultimate fate of the yolk stalk in the chicken, there is contradictory evidence indicating that it disappears within the first 5 months of life (*Maumus, 1902*) and that it persists in the adult bird (*Latimer, 1924*) as a functional part of the intestine (*Kar, 1947*). It is said to remain permanently in *Anseriformes* and other water birds and has been observed in many adult passerine birds.

### THE AMNION AND THE CHORION

The amnion is a liquid-filled sac that surrounds the embryo and performs a predominantly protective function. Its contained fluid gently supports the embryo, prevents the dehydration of its tissues, insulates it against sudden changes in temperature, and reduces the impact of mechanical shocks, thus lessening the possibility of injury. The amnion itself, though thin, is very elastic. It is composed in part of smooth muscle, the irregularly rhythmical contractions of which keep the contents of the sac in motion and prevent the membrane from adhering to the embryo. In addition, the amniotic cavity serves as a channel for the utilization of the egg albumen, which the embryo swallows with the amniotic fluid during the late stages of incubation.

The amnion develops simultaneously with the chorion from the reduplicated extraembryonic part of the blastoderm. The chorion is external to the amnion and is separated from it by a space, the extraembryonic coelom or exocoelom. Initially without apparent functional significance, the chorion becomes of utmost physiological importance when the allantois, invading the exocoelom, fuses with it to form the chorioallantois.

Because of the manner of their formation, both the amnion and the chorion consist of an ectodermal and a mesodermal layer whose relationship to each other is reversed in the two membranes. Thus ectoderm is external to mesoderm in the chorion but comprises the internal layer of the amnion. The ectoderm of the amnion is continuous medially with the ectoderm of the embryonic body. The outer (mesodermal) layer of the amnion differentiates as muscle and, like the mesoderm of the chorion, eventually fuses with the mesoderm of the allantois. The participation of mesoderm in the development of the amnion was first recognized by Remak (1855),



who corrected the earlier impression (*Baer, 1828b*) that the membrane was formed of ectoderm alone.

### The Formation of the Amnion and the Chorion

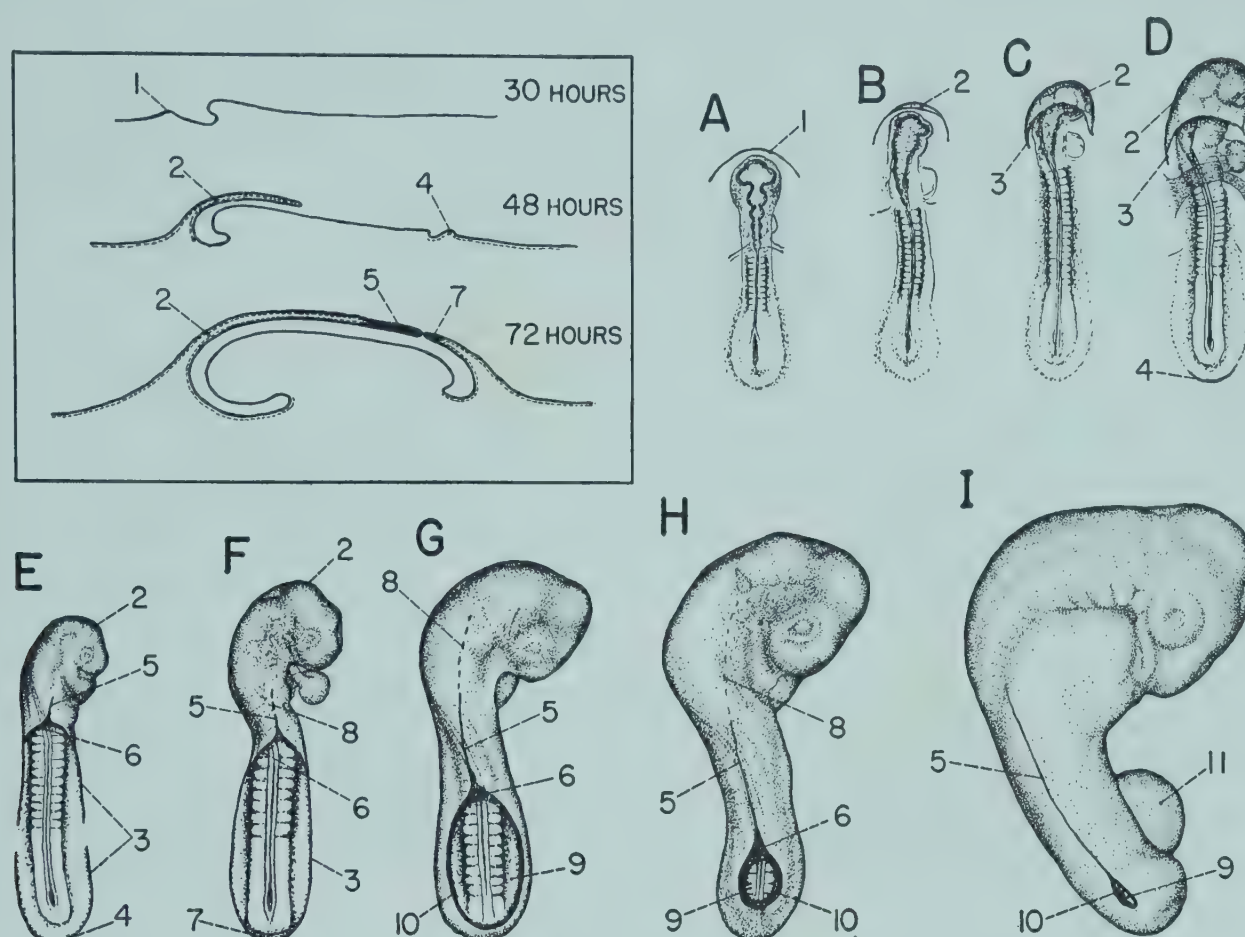
The amnion and the chorion are derived from the extraembryonic somatopleure (*Remak, 1855*), that is, from the somatopleure which is not folded off into the body wall of the embryo. The development of pieces of the head-process chick blastoderm transplanted to the chorioallantois indicates that the membrane-forming material is coextensive with the lateral mesoderm (*Kumé, 1951*). The amnion arises from the part of the extraembryonic somatopleure which is nearest the embryo, and the chorion from the remaining, more peripheral portion. The process of amnion and chorion formation consists essentially of the evaluation of the somatopleure into folds at the junction of the amniogenous and choriogenous areas and the growth of these folds over the embryo from either side, beginning anteriorly. When the embryo is completely covered, the somatopleuric reduplication is transformed into two concentric membranes, the amnion and the chorion, which are separated by the exocoelom everywhere except in a small region representing a remnant of the line of fusion between the folds. This region is known as the sero-amniotic connection ("serosa" being the older term for the chorion) and is of considerable physiological importance.

### The Amniotic Folds

The folding of the somatopleure is always preceded by a thickening of the ectoderm at the boundary between the amniogenous and the choriogenous area, that is, along the line representing the apex of the future elevation (*Weber, 1902f, 1903; Lillie, 1903*). This thickening, which has been termed the ectamnion (*Lillie, 1903*), is first seen at the stage of about 9 somites in the form of a crescent lying anterior and anterolateral to the embryo's head (Fig. 397) and gradually fading out at the level of the heart (*Lillie, 1903*). About 10 hours later, that is, by the 40-hour (13-somite) stage (*Hirota, 1894*), this arc of ectamnion occupies the apex of the first definitely recognizable amniotic fold, which is known as the head fold of the amnion. The head fold appears at the 13-somite stage in the European lapwing (*Vanellus vanellus*), also (*Grosser and Tandler, 1909*), and it has formed in the European coot (*Fulica atra*) by the 16-somite stage (*Steinmetz, 1930*). The head has now begun to sink down toward the yolk, a circumstance which *Shore and Pickering (1889)* regarded as the sole cause of the elevation of the amniotic head fold. However, there is marked cellular proliferation in the ectamnion (*Weber, 1903*), and the cells of the ectamnion are distinctly different in appearance from the adjacent ectodermal cells (*Adamstone, 1948*). It is probable that the ectamnion forms through a process of self-differentiation (*Lillie, 1903*). It is also probable



that it is the presence of the ectamnion, rather than purely mechanical factors, which causes the elevation of the head fold, for the head fold fails to form if the ectamnion of the 30-hour chick is destroyed by cautery. Cauterization of the blastoderm lateral to the body has no effect on amnion formation (Adamstone, 1948).



**Fig. 397.** Successive stages in the development of the amnion of the chick embryo. (Drawn by the author from various sources.)

*Insert:* diagrammatic longitudinal sections; ectoderm represented by solid line, mesoderm by broken line.

Surface views at the following stages: A, 9 somites, 29 hours; B, 14 to 15 somites, 34 hours; C, 18 somites, 37 hours; D, 22 somites, 42 hours; E, 27 somites, 48 hours; F, 32 somites, 54 hours; G, 34 to 35 somites, 60 hours; H, 37 somites, 66 hours; I, 39 somites, 72 hours. All  $\times 5$ .

1, ectamnion preceding amniotic head fold; 2, amniotic head fold; 3, lateral amniotic fold; 4, ectamnion preceding amniotic tail fold; 5, sero-amniotic connection; 6, thickened ectoderm at posterior edge of amniotic head fold; 7, amniotic tail fold; 8, median mesodermal septum between coelomic cavities; 9, amniotic umbilicus; 10, thickened ectoderm surrounding amniotic umbilicus; 11, allantois.

In the chick, the amniotic head fold appears at, or somewhat before, the time that the anterior horns of the mesoderm complete their pincers-like movement and meet in the midline of the blastoderm anterior to the embryo's head. The union of the mesodermal horns leaves the oval proamnion (Fig. 398-A and B) still free of mesoderm. Except for its anterior end, the proamnion lies beneath the projecting head and is pushed down as the latter sinks yolkward. The most cephalic part of the amniotic head fold forms just within the anterior limit of the proamnion (cf. Fig. 398-B). In



the beginning, therefore, the head fold consists of ectoderm and endoderm. In the chick, however, this initial stage is of very brief duration.

At the 14- to 15-somite stage (Fig. 398-C and D), the embryo's head has sunk considerably lower, and its anterior tip is covered by the amniotic head fold (Shore and Pickering, 1889; Hirota, 1894; Lillie, 1903). At first, the backward growth of the head fold is the result of fusion in the midline,

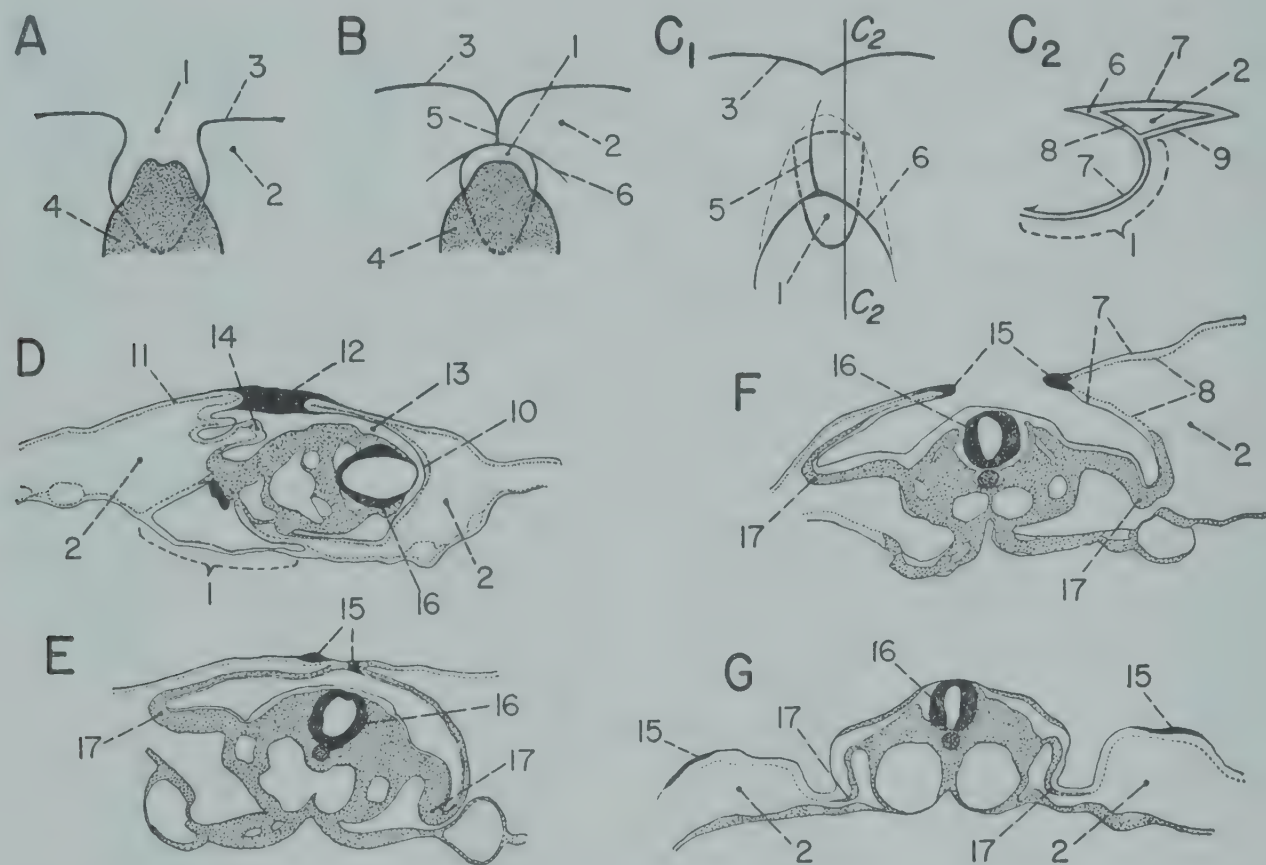


Fig. 398. Early stages in the formation of the amnion. (Redrawn with modifications A, B, C<sub>1</sub>, C<sub>2</sub>, D, after Hirota, 1894; E, F, G, after Weber, 1903.)

A, B, C<sub>1</sub>, diagrams representing surface views of the chicken blastoderm after approximately 27, 37, and 42 hours' incubation, respectively (the head is omitted in C<sub>1</sub>); C<sub>2</sub>, diagram representing a section along the line indicated in C<sub>1</sub>; D, semidiagrammatic representation of a cross section through the cephalic portion of a 48-hour chicken blastoderm; E, section through the anterior trunk region of a 78-hour duck (*Anas platyrhynchos*) blastoderm at the level where the lateral amniotic folds are about to fuse ( $\times 25$ ); F, a section of the same blastoderm taken more posteriorly ( $\times 25$ ); G, section of a 70-hour duck blastoderm a considerable distance below the level where the amniotic folds have met in the midline ( $\times 25$ ).

1, proamnion; 2, exocoelom; 3, sinus terminalis; 4, head; 5, mesodermal septum separating the right and left halves of the exocoelom; 6, amniotic head fold; 7, ectoderm; 8, mesoderm; 9, endoderm; 10, amnion; 11, chorion; 12, sero-amniotic connection; 13, amniotic cavity; 14, secondary amniotic fold; 15, ectamnion at apex of lateral amniotic fold; 16, neural tube; 17, lateral limiting sulcus.

of the ectamnion from right and left, a process which, progressing caudad, forms a shallow pocket for the head (Lillie, 1903). The mesoderm, split into two layers separated by the extraembryonic coelom, is now drawn into the amniotic head fold. This initial penetration of mesoderm occurs at the same stage in the European lapwing, *Vanellus vanellus* (Grosser and Tandler, 1909). A midsagittal section of the blastoderm shows that the



amniogenous limb of the fold now consists of ectoderm and mesoderm from the apex of the fold to the anterior boundary of the proamnion, where the mesoderm is reflected peripherally as the splanchnic mesoderm (cf. Fig. 398-D). The median mesodermal septum that separates the right and left coelomic cavities has also been prolonged into the head fold but is beginning to disappear peripherally (cf. Fig. 398-C), near the sinus terminalis (*Hirota, 1894*).

The head fold rapidly extends itself posteriorly by the same process of fusion that started its caudad growth (Fig. 398-E, F, and G). The ectoderm of the leading edge always progresses slightly in advance of the inner mesodermal component of the fold. In the 48-hour chick, the head fold has attained or passed the level of the heart (cf. Fig. 397). A similar stage of development is seen in the 115-hour embryo of the emu, *Dromaeus novae-hollandiae* (*Haswell, 1887*). The head fold covers the embryo like a sac open posteriorly. The ends of its curved free edge continue posteriorly for a short distance as the lateral amniotic folds (cf. Fig. 398-G) which are elevated as the result of traction exerted on the somatopleure by the head fold (*Lillie, 1903; Adamstone, 1948*). Except in the region of the proamnion, the head fold now consists of four layers, an outermost and an innermost layer of ectoderm and two middle layers of mesoderm. It is now clear that mesoderm will form the inner layer of the chorion and the outer layer of the amnion and that ectoderm will comprise the remaining layer of each membrane.

In the middle of the free posterior margin of the head fold, fusion of the ectamnion from right and left has produced a mass of ectodermal cells, triangular in surface view (cf. Fig. 397-I). This mass of cells forms a direct link between the ectoderm of the chorion and that of the amnion and constitutes the sero-amniotic connection. In cross section, the ectodermal mass is seen to be biconcave (cf. Fig. 398-E) because of the bilateral encroachment of the folds of mesoderm marking the median boundaries of the right and left coelomic cavities. These folds meet anterior to the ectodermal mass to form the portion of the median mesodermal septum which disappears later. With the continued growth of the head fold, the sero-amniotic connection is prolonged as a narrow ectodermal suture, or raphe, inserted between the two folds of mesoderm (*Schenk, 1871; Hirota, 1894*).

The embryo begins to turn on its left side shortly after the amniotic head fold appears. The sero-amniotic raphe, as it forms, does not turn with the body but remains above it, over its right side rather than dorsal to it. Rotation of the body around its longitudinal axis therefore exerts a strong tension on the somatopleure of the left side, and the inner limb of the fold is stretched tautly around the embryo. On the right side, however, the inner limb of the fold is relatively loose, and secondary folds appear in it temporarily (*Lillie, 1903*), as may be seen in Fig. 398-E.



While the four-layered amniotic head fold is growing down over the dorsal surface of the embryo, the folds of mesoderm enclosing the right and left coelomic cavities anteriorly are extending beneath the embryo's head and are encroaching upon the proamnion from either side. The proamnion thus becomes continually narrower. Its invasion by mesoderm is indicated by the gradual migration of the anterior vitelline veins toward the midline, for these veins lie very close to the lateral boundaries of the proamnion (Ravn, 1886). In the chick, the proamnion is virtually obliterated at some

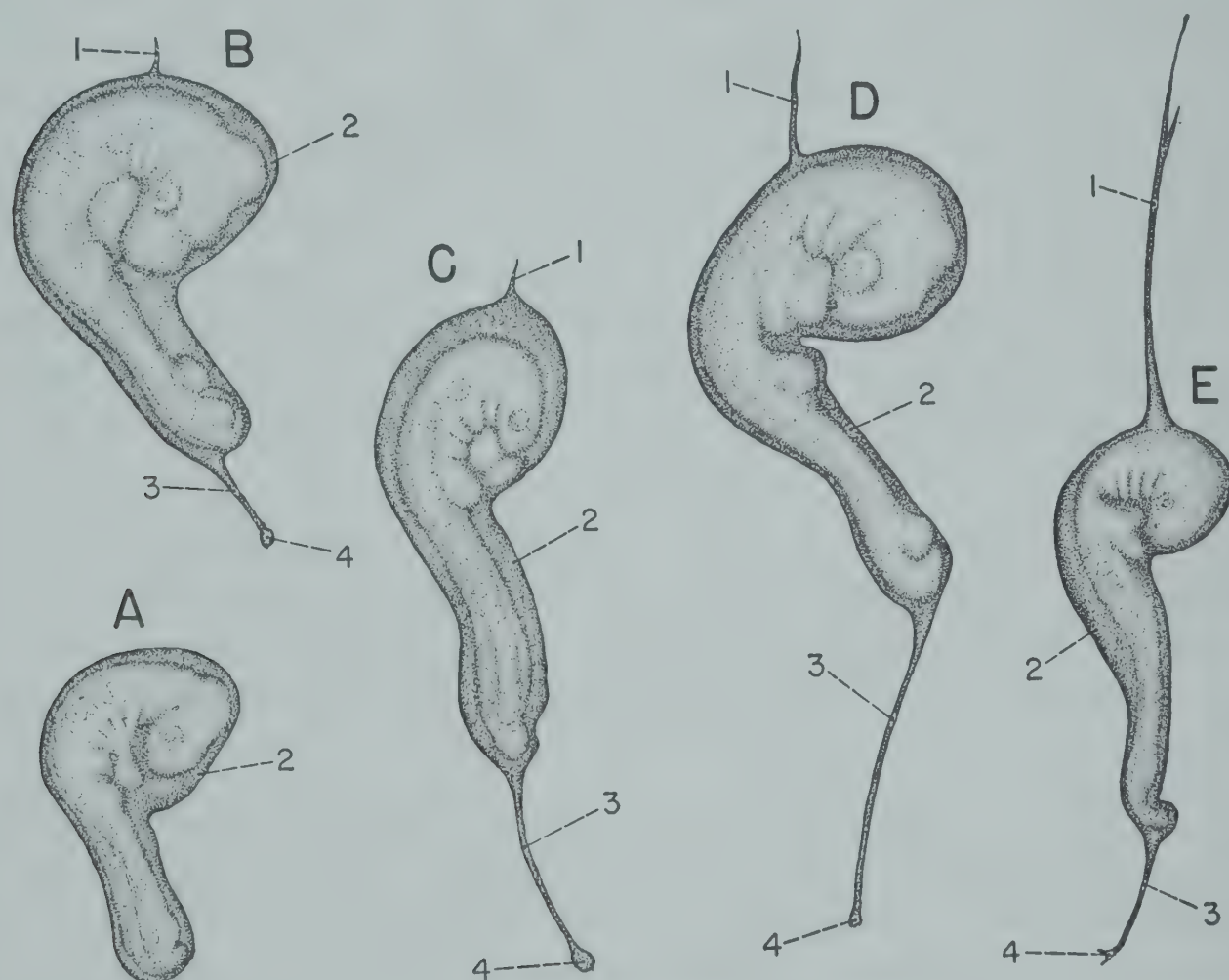


Fig. 399. Various forms of amniotic appendages in birds.

A, amnion without appendages, in chicken, *Gallus gallus* (redrawn from the author's collection); B, C, D, and E, amnion with anterior and posterior appendages: in the coot, *Fulica atra*, redrawn after Steinmetz (1930); and in the shearwater, *Puffinus pacificus*, in the tropic bird, *Phaëthon rubricauda*, and in the albatross, *Diomedea immutabilis*, redrawn after Schauinsland (1903b).

1, anterior prolongation of amnion; 2, amnion; 3, caudal amniotic canal; 4, amniotic infundibulum.

time between the sixtieth and the sixty-eighth hour of incubation (Ravn, 1886; Shore and Pickering, 1889). The final stages of its disappearance involve the formation and rupture of a median mesodermal septum between the right and left coelomic cavities. A blind ectodermal pocket at the posterior end of the proamnion (where its ectoderm is reflected anteriorly into the ectoderm of the body) grows backward to the level of the liver and is found in the midst of the ventral liver mesentery before being gradually incorporated into the amniotic cavity (Ravn, 1886).



In a number of birds, the closure of the anterior mesodermal horns seems to occur much more slowly than in the chick, and, in relation to mesoderm growth, amnion formation is earlier (Schauinsland, 1903b, 1906). The formation of the amniotic head fold is somewhat precocious in the starling (*Sturnus vulgaris*), the sparrow (*Passer domesticus*), and the crow (*Corvus cornix*), and is very precocious in such water birds as terns (*Sterna* sp.), the albatross (*Diomedea immutabilis*), the shearwater (*Puffinus pacificus*), the booby (*Sula sula*), the tropic bird (*Phaëthon rubricauda*), the frigate bird (*Fregata aquila*), and the godwit (*Limosa lapponica*). In the tropic bird and the albatross, the amniotic head fold appears long before the horns of the mesoderm come together, and it consists of ectoderm and endoderm until it has covered the entire head; mesoderm then grows in from either side and displaces the endoderm. When the free edge of the head fold has reached the level of the omphalomesenteric arteries, the proamnion is very small in the chick, but it is still large in the albatross and the sooty tern (*Sterna fuliginosa*).

In some of the water birds that he studied, Schauinsland (1903b, 1906) observed a narrow, tubular anterior prolongation of the amnion (Fig. 399) which arises because the most anterior portion of the amniotic ectoderm remains suspended between the dorsal and ventral ends of the median mesodermal septum separating the right and left coelomic cavities (Fig. 400-A and B). The growth of the blastoderm elongates this extension of the amnion to varying degrees in the albatross (*Diomedea immutabilis*), tropic bird (*Phaëthon rubricauda*), the sooty tern (*Sterna fuliginosa*), the common tern (*Sterna hirundo*), and the booby (*Sula sula*). In the European coot (*Fulica atra*), the structure is quite small (Steinmetz, 1930).

At the end of the second or the beginning of the third day of the chick's incubation period, the ectamnion can be traced far caudad from the ends of the short lateral amniotic folds. Behind the tail, the incipient amniotic tail fold (cf. Fig. 397) is seen as a crescent of ectamnion with concavity directed anteriorly (Hirota, 1894; Lillie, 1903).

The tail fold, which contains a mesodermal component even in its initial stages, now begins to grow cephalad over the embryo's tail, though progressing at a much slower rate than the head fold. The initial development of the tail fold has been observed in the 27-somite embryo of the Emperor penguin, *Aptenodytes forsteri* (Glenister, 1954). The tail fold seems to represent an independent center of amnion formation (Lillie, 1903), for, if the appearance of the head fold is prevented by cauterization, the tail fold may nevertheless arise and extend itself anteriorly until its further progress is inhibited by the flexure of the body (Adamstone, 1948).

As the tail fold increases in size, its anterior prolongations become continuous with the posterior prolongations of the head fold, and the lateral amniotic folds thus come into prominence, connecting the head fold and



the tail fold. The lateral folds rise high over the embryo's body on either side as the head fold and tail fold approach each other from opposite directions. Consequently, there is formed an elliptical opening, the "amniotic umbilicus," surrounded by the amniotic folds. This opening becomes continually smaller as the folds grow centripetally (cf. Fig. 397). It is soon

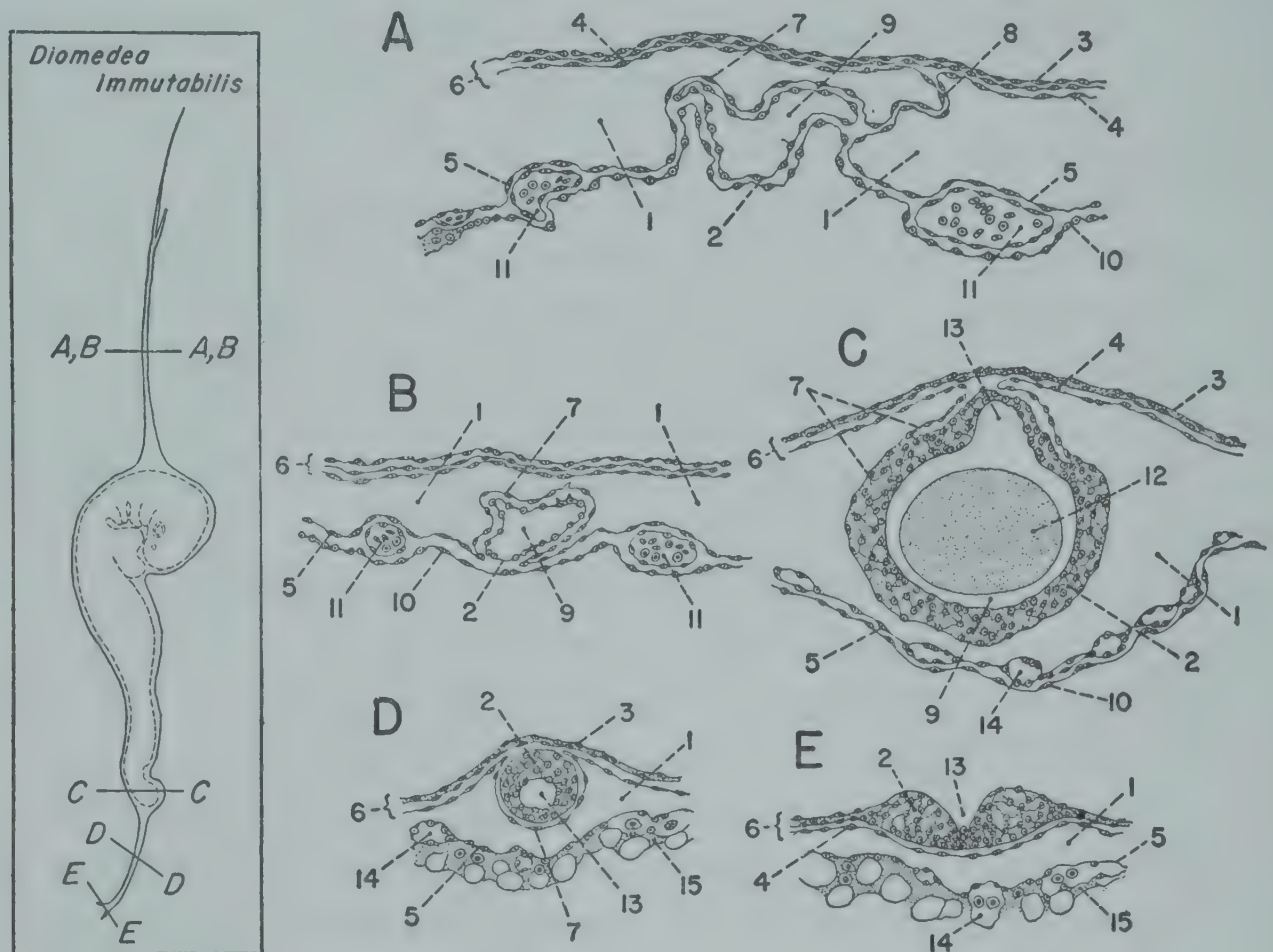


Fig. 400. Cross sections through the anterior and posterior prolongations of the amnion of the albatross, *Diomedea immutabilis*. (Redrawn with modifications after Schauinsland, 1903b.)

A, section of the anterior prolongation at a time when it is suspended from the chorion by a dorsal mesodermal mesentery; B, same section at a later stage, after the rupture of the dorsal mesentery and shortly before the formation of the temporary ventral mesentery; C, section through the caudal end of the embryo in the region where the caudal amniotic canal takes its origin; D, a more posterior section through the middle of the amniotic canal; E, section posterior to D, at the point where the caudal amniotic canal comes to the surface of the blastoderm, and its ectoderm is continuous with the ectoderm of the chorion. All  $\times 80$ .

Insert: shows location of cross sections.

1, exocoelom; 2, ectoderm of amnion; 3, ectoderm of chorion; 4, somatic mesoderm; 5, splanchnic mesoderm; 6, chorion; 7, mesoderm of amnion; 8, dorsal mesodermal mesentery; 9, amniotic cavity; 10, endoderm; 11, right and left anterior vitelline arteries; 12, body of embryo; 13, caudal amniotic canal; 14, blood vessel; 15, yolk sac endoderm.

edged with a ring of greatly thickened ectoderm which includes the triangular mass of cells anteriorly and an irregular proliferation of tail fold ectoderm posteriorly. At the end of the chick's third incubation day, or early in the fourth day, the margins of the amniotic umbilicus coalesce at the level of the leg buds, and the opening thus disappears (Hirota, 1894).



Closure occurs at the approximately equivalent stage of 38 somites in the embryo of the Emperor penguin, *Aptenodytes forsteri* (Glenister, 1954). A very small amniotic umbilicus is still present in the 7-day emu (*Dromaeus novae-hollandiae*) embryo (Haswell, 1887).

In some birds, the tail fold and the lateral folds are small or even absent entirely, and closure of the amniotic umbilicus does not proceed in the manner described above. Instead, the head fold merely grows down over the tail bud, becoming narrower as it completes the envelopment of the caudal end of the body. It then continues to grow distally, and the amniotic umbilicus is elongated to form a tube or canal of thickened ectoderm (cf. Fig. 400) which is finally closed with an ectodermal plug. Schauinsland (1906) noted that the length of this posterior appendage of the amnion is inversely proportional to the size of the amniotic tail fold. Thus a very small tail fold and an extremely long canal are found in the shearwater (*Puffinus pacificus*), whereas a more pronounced tail fold and a shorter canal occur in the herring gull (*Larus argentatus*) and the starling (*Sturnus vulgaris*). The caudal amniotic canal is present also in the albatross, *Diomedea immutabilis*, the tropic bird, *Phaëthon rubricauda*, the common and sooty terns, *Sterna hirundo* and *Sterna fuliginosa* (Schauinsland, 1903b), and the European coot, *Fulica atra* (Steinmetz, 1930), all of which possess an anterior prolongation of the amnion (cf. Fig. 399). Grosser and Tandler (1909) mentioned the presence of the caudal canal in the European lapwing (*Vanellus vanellus*), which, like the coot, does not develop an amniotic tail fold. It is of interest to note that the amniotic tail fold does not form in the chick if the blastoderm posterior to the tail is cauterized at the end of the second day of incubation. The amniotic head fold then grows down over the entire body, and the amnion closes behind the tail, around the site of cautery. The tail fold, therefore, is not essential to the formation of the chick's amnion (Adamstone, 1948).

After the closure of the amniotic sac, the membrane not only expands to accommodate its fluid contents, but undergoes actual growth, as indicated by an increase in weight. This increase is most marked between the ninth and the fourteenth days of the chick's incubation period (Fig. 401). Little or no growth occurs during the last week of development.

As the embryo's body becomes clearly separated from the extraembryonic tissues, the portion of the amnion which is directly continuous with the embryo becomes increasingly constricted. By the end of the chick's sixth incubation day (Duval, 1889), this portion is transformed into a tubular structure, the somatic stalk, which encloses the stalks of the yolk sac and the allantois. At hatching, all the amnion is discarded except part of its stalk (Virchow, 1891).

**Suppression of the amnion.** Total absence of the amnion sometimes occurs spontaneously. Several anamniote chick embryos were observed by



Darceste (1879), who reported that most of them were compressed by the vitelline membranes or the shell. The result was either anomalous development, or rupture of the vessels of the area vasculosa followed by death. One anamniote embryo, however, was alive and apparently normal on the thirteenth day of incubation.

The formation of the amnion has also been prevented experimentally. Completely anamniote chick embryos have been produced by cautery in the proamnion and tail fold regions before the amniotic folds arise (Adamstone, 1948). Ancel and Lallemand (1943*a*, 1943*b*) found that the develop-

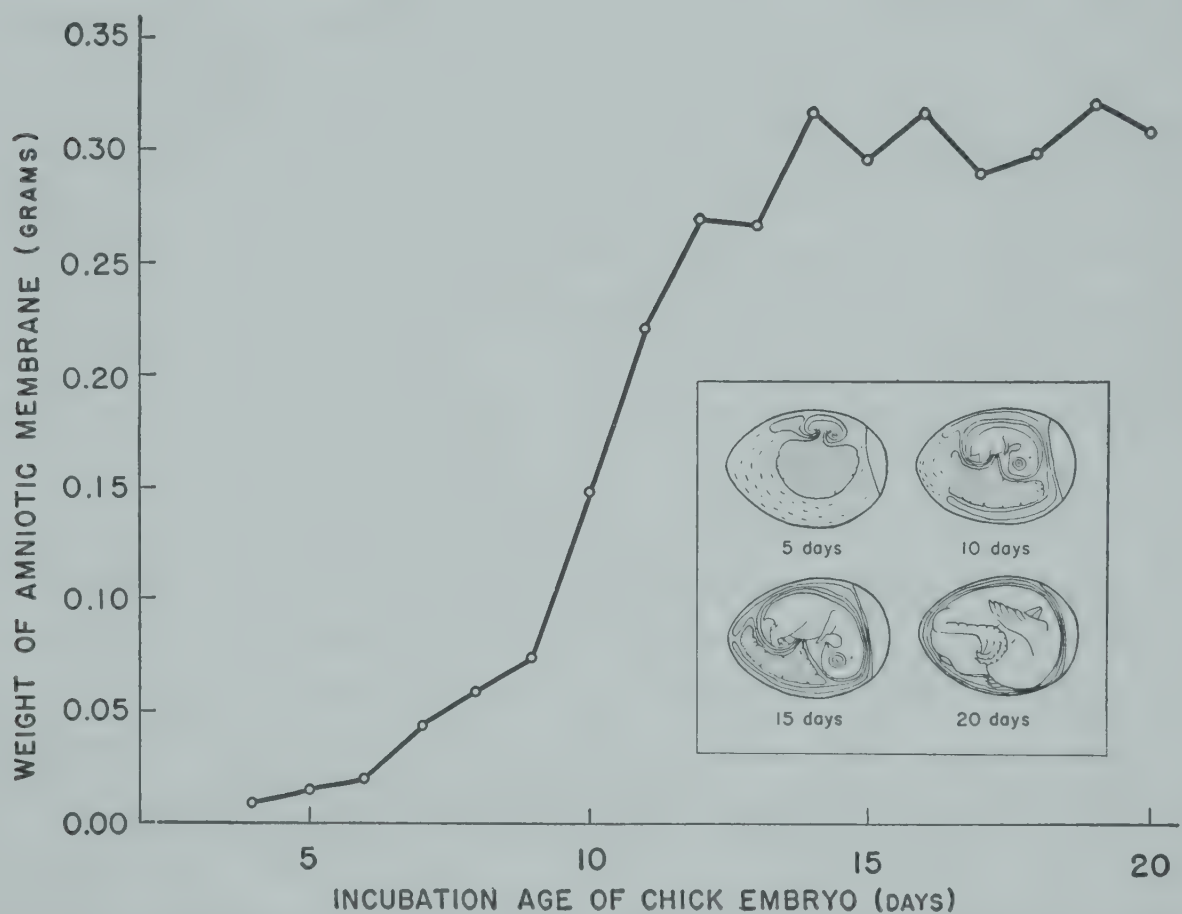


Fig. 401. Weight of amniotic membrane of chick (*Gallus gallus*) showing greatest increase between ninth and fourteenth day of incubation. (After Romanoff, unpublished.)

*Insert:* a diagrammatic presentation of the amnion in relation to surrounding structures at several stages of embryonic development.

ment of the amnion could frequently be arrested (or limited to head fold formation) by injecting the egg, at the end of the second day of incubation, with an appropriate quantity of abrin, ricin, trypanflavin, testosterone propionate, progesterone, or desoxycorticosterone acetate. In the absence of the amnion, the embryo remained spread out flat on the blastoderm for an unusually long time, and the closure of the thoracoabdominal wall was retarded. Survival was limited, although the maximum age at death varied with the different drugs. The tabulated data summarize some of the results obtained with these five substances (which were alone effective out of more than seventy used).



Substance	Optimal Dose (mg.)	Frequency of Anamniote Embryos (per cent)	Maximum Age at Death (days)
Abrin	0.002	28.5	6
Ricin	0.000175	40.9	7
Testosterone propionate	2.5	40.0	7
Progesterone	0.5	64.0	7
Desoxycorticosterone acetate	0.125	60.0	15
Trypaflavin	0.005	45.0	21

### *The Secondary Sero-Amniotic Connection*

The ectoderm of the sero-amniotic connection gradually disappears (*Schenk, 1871*) and is replaced by mesoderm (*Hirota, 1894*). The removal of the ectodermal raphe is accomplished by the insinuation of mesodermal tissue, accompanied, probably, by degeneration of the ectodermal cells (*Schenk, 1871; Weber, 1902f, 1903*). As previously mentioned, the encroachment of mesoderm starts as soon as the sero-amniotic connection forms; and it continues after the amniotic umbilicus has closed. During the chick's fourth incubation day, mesoderm from one side begins to pierce the ectodermal bridge in places and to unite with mesoderm of the opposite side (Fig. 402-A and A<sub>2</sub>). On the sixth day, the ectodermal raphe is discontinuous anteriorly, and it has been completely superseded by a mesodermal reticulum by the ninth or tenth day. This network of mesoderm now intervenes between the ectodermal layers of the amnion and the chorion and constitutes the secondary, mesodermal, sero-amniotic connection (*Hirota, 1894*).

The secondary sero-amniotic connection is wider from the beginning than the original, ectodermal structure. Its width is further increased by the coalescence of the amniotic and chorionic mesoderm for a considerable distance to right and left, so that the entire connection is transformed into a plate (Fig. 402-B<sub>1</sub>) by the tenth or twelfth day (*Hirota, 1894*). This platelike connection, of course, consists of three layers: two of ectoderm (amnionic and chorionic) with an intermediate layer of mesoderm (fused amnionic and chorionic).

Almost as soon as it has formed, the secondary sero-amniotic connection begins to break down (Fig. 402-B<sub>2</sub> and B<sub>3</sub>). Perforations appear in it on the chick's eleventh day, the sparrow's (*Passer domesticus*) ninth (*Witschi, 1949*). The openings gradually become larger and coalesce, so that only a few strands of tissue remain between them (Fig. 402-C, D, and E). The perforations of the plate establish a communication between the amniotic cavity and the cavity of the albumen sac, which lies on the other side of the sero-amniotic plate. As Maître-Jean (1722) suspected but could not prove, albumen now begins to enter the amniotic cavity and to mingle with the amniotic fluid (*Hirota, 1894; Fülleborn, 1895*).



The rupture of the sero-amniotic plate is delayed by 24 hours or more in chicken eggs that are not turned during incubation (*Randles and Romanoff, 1949, 1950*). Experimental evidence also indicates that perforation is retarded when the incubation temperature is subnormal and hastened when the temperature is elevated (*Romanoff, 1952*).

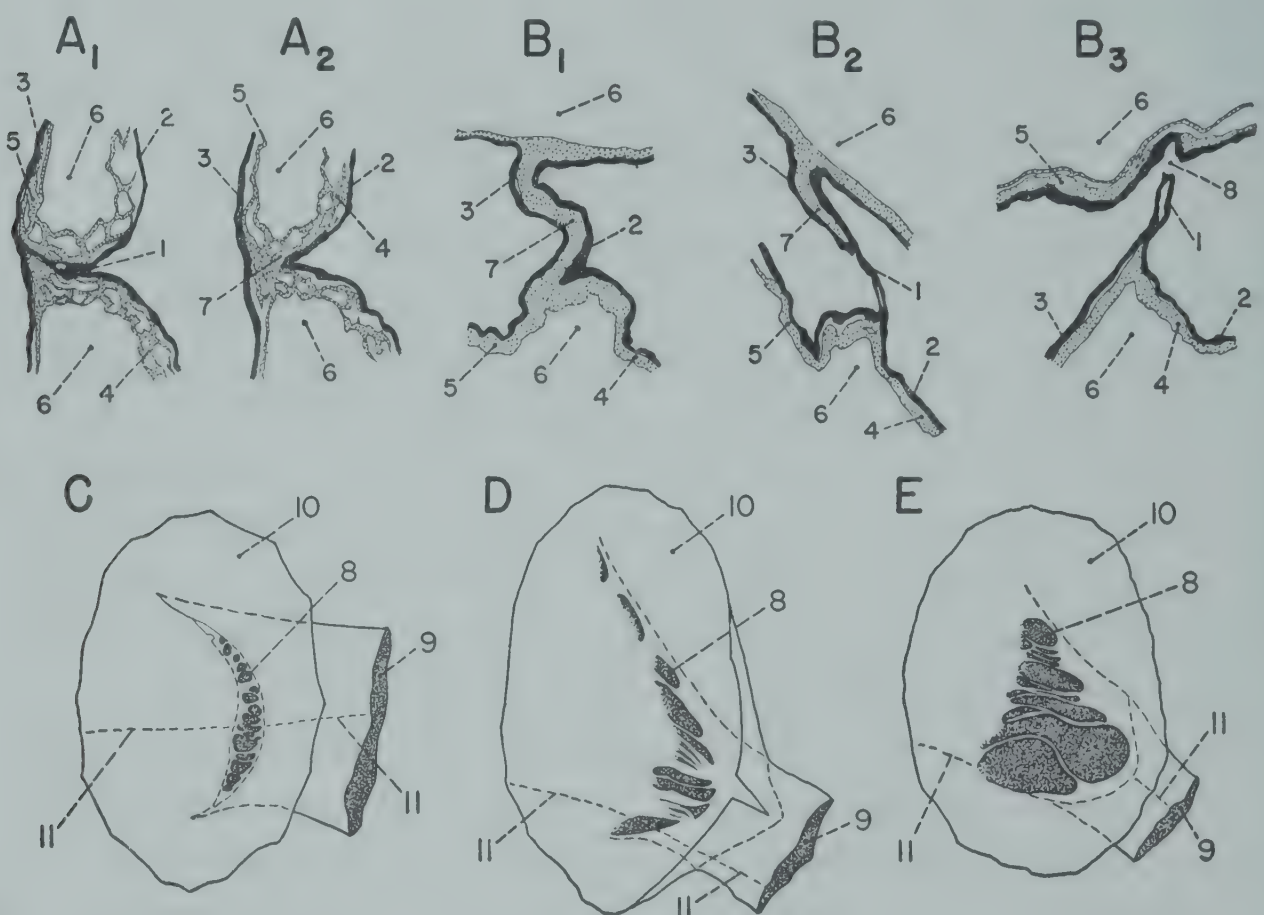


Fig. 402. The formation and perforation of the secondary (mesodermal) sero-amniotic connection in the chicken egg. (Redrawn with modifications after Hirota, 1894.)

A<sub>1</sub>, A<sub>2</sub>, cross sections at the 4.5-day stage taken, respectively, at a level where the ectodermal connection is still intact, though thin, and at a level where it has been replaced by mesoderm growing in from either side (both  $\times 15$ ); B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, cross sections at the 12-day stage taken, respectively, at levels where the sero-amniotic connection is a three-layered plate, where it consists in part of a thin ectodermal sheet, and where this sheet has become perforated (all  $\times 15$ ); C, D, E, diagrammatic representations of the appearance of the perforated sero-amniotic connection as seen from within the amniotic cavity after 14, 15, and 17.5 days of incubation, respectively (all  $\times 3$ ).

1, ectoderm of sero-amniotic connection; 2, ectoderm of amnion; 3, ectoderm of chorion; 4, mesoderm of amnion; 5, mesoderm of chorion; 6, exocoelom; 7, mesoderm of sero-amniotic connection; 8, perforation in sero-amniotic connection; 9, albumen sac; 10, portion of amnion; 11, line along which the interallantoic septum is continuous with the inner limb or wall of the allantois.

### Histological Differentiation in the Amnion

The inner ectodermal layer of the amnion soon forms an epithelium and undergoes little further differentiation. The outer mesodermal layer, however, gives rise to smooth muscle disposed in a very distinctive manner. Fibers that appear to have a supporting function have also been observed.



### The Epithelium

The ectodermal component of the amnion has a more or less syncytial structure before the amniotic umbilicus closes (Fig. 403). Soon afterward (that is, at the end of the chick's third incubation day), cell boundaries start to appear. The polygonal outlines of individual cells are easily recognizable at the beginning of the next day (Pierce, 1933), and treatment with silver nitrate demonstrates the presence of intercellular cement (Peterfi, 1913; Lewis, 1923b). The ectoderm now consists of a single layer of flat epithelial cells about 20 microns in diameter, each with a round to oval,

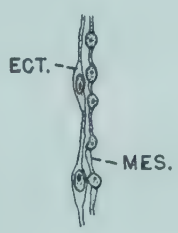
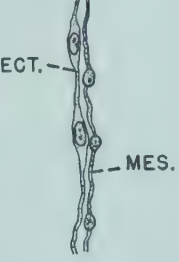
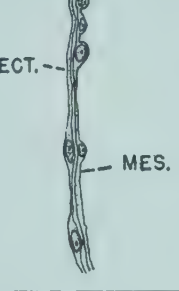
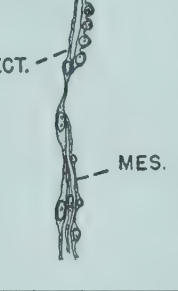
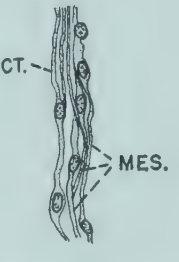
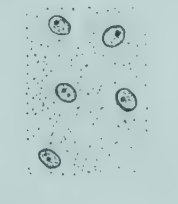

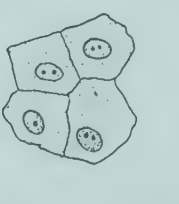

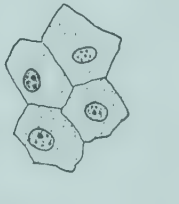
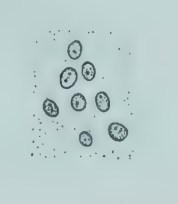
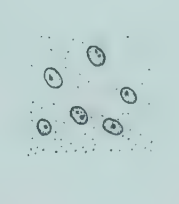
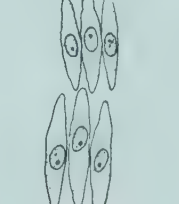
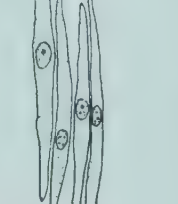

STRUCTURE OF AMNION AT VARIOUS STAGES OF INCUBATION					
	48-65 HOURS	72 HOURS	96 HOURS	7 DAYS	12 DAYS
AMNIOTIC WALL (CROSS SECTION)					
ECTODERM (SURFACE VIEW)					
MESODERM (SURFACE VIEW)					

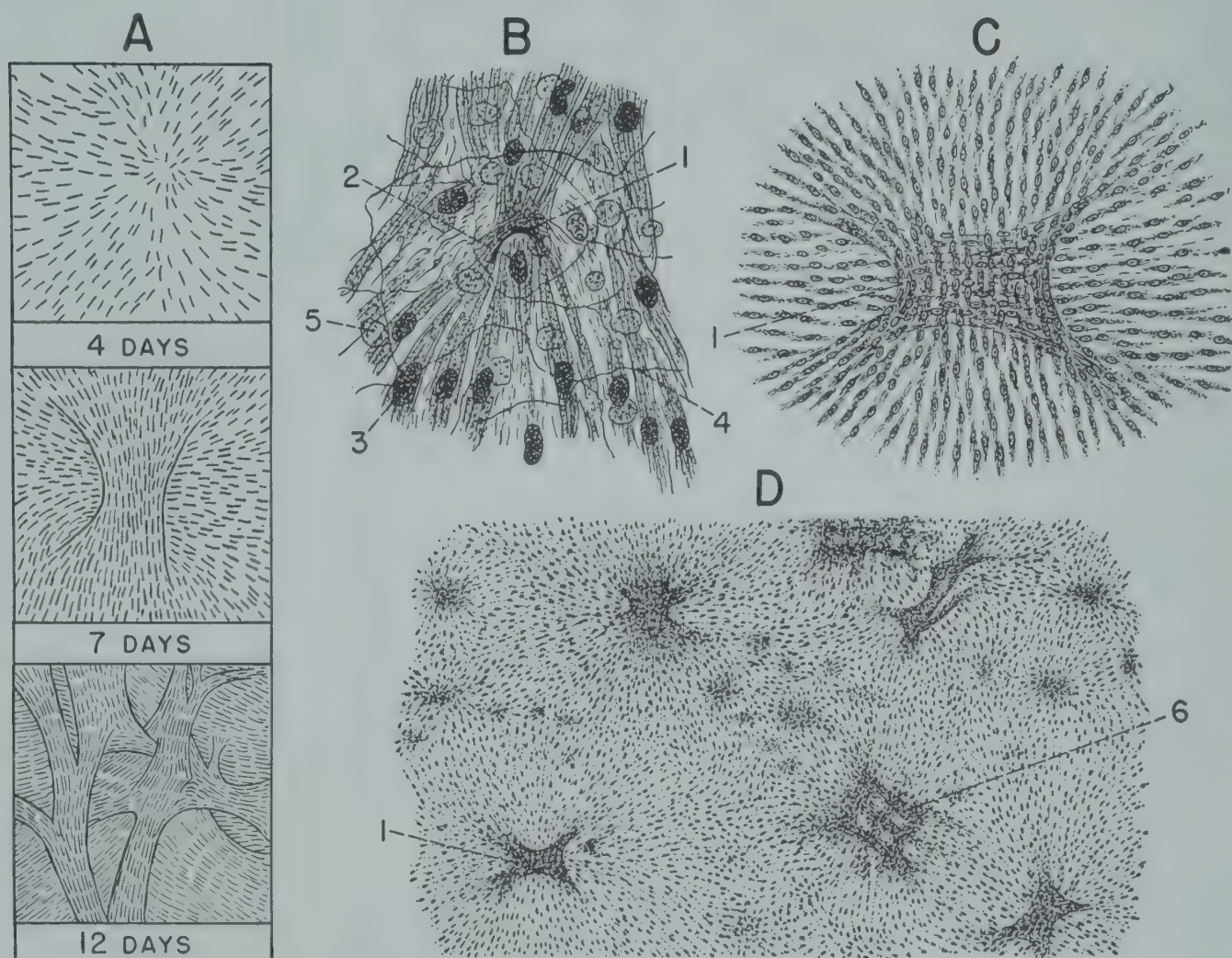
Fig. 403. Successive stages in the structural development of the chick amnion. (Redrawn with rearrangements after Pierce, 1933.)  $\times 250$ .

Ect., ectoderm; Mes., mesoderm.

slightly eccentric nucleus, one or two (occasionally more) nucleoli, clear or finely reticulated cytoplasm, and filamentous mitochondria concentrated near the nucleus (Peterfi, 1913; Lewis, 1923b; Pierce, 1933; Bautzmann and Schröder, 1953). Lewis (1923b) was unable to find centrioles in cultured amniotic epithelial cells but observed that fat globules were fairly common. A distinguishing feature of these cells is the presence of vacuoles, which deform the nuclei and increase in number and size until the eighth or tenth day (Peterfi, 1913; Trotti, 1933b; Bautzmann and Schröder, 1953). Histochemical reactions show that the vacuoles contain lipoids (Trotti, 1933b), probably confined to a perivacuolar position; the interior of the vacuoles may consist of amniotic fluid (Bautzmann and Schröder, 1953).



It has been said that fibrils condense from the limiting membranes of the vacuoles (*Peterfi, 1913*), but this statement has been questioned (*Bautzmann and Schröder, 1953*). Trotti (*1933b*), whose observations extended to the seventeenth day, reported that the epithelial cells of the amnion gradually increase in height until they are transformed into cylindrical elements.



**Fig. 404.** Arrangement of mesodermal cells of amnion of the chick. (Redrawn with modification A, after *Pierce, 1933*; B, C, and D, after *Verzar, 1908*, from preparations colored with haematoxylin and eosin.)

A, semidiagrammatic drawings of the surface views showing the arrangement of mesodermal cells of the amnion at different stages of development: at 4 days, showing the starlike arrangement; at 7 days, showing the beginning of a cross over figure; and at 12 days, showing the complicated cross over figure; B, arrangement of mesodermal cells of the amnion of 5 days, showing wide-meshed network of epithelial cells and epithelial nuclei ( $\times 170$ ); C, arrangement of mesodermal cells of the amnion of 7 days 8 hours ( $\times 65$ ); D, typical and atypical arrangement of mesodermal cells of the amnion of 7 days 8 hours ( $\times 35$ ).

1, parabolic fibrils; 2, primary center cell; 3, secondary inosculating cell; 4, epithelial cell boundary; 5, epithelial nucleus; 6, developing parabolic figure.

### *The Musculature*

Until the end of the chick's third day of incubation, the mesodermal layer of the amnion is a syncytium (cf. Fig. 403) with small oval nuclei each containing a single nucleolus. Around the umbilicus (the region of attachment of the membrane to the embryo), the long axes of the nuclei



are arranged radially. Elsewhere, the cells have formed condensations connected by fine strands of cytoplasm, which may be either cell processes or thick, primitive muscle fibrils (Fig. 405). During the course of the fourth day, these cytoplasmic strands constitute a meshed network the interstices of which gradually grow narrower and finally close up. At the same time, cell boundaries become apparent. The cells are spindle-shaped (cf. Fig.

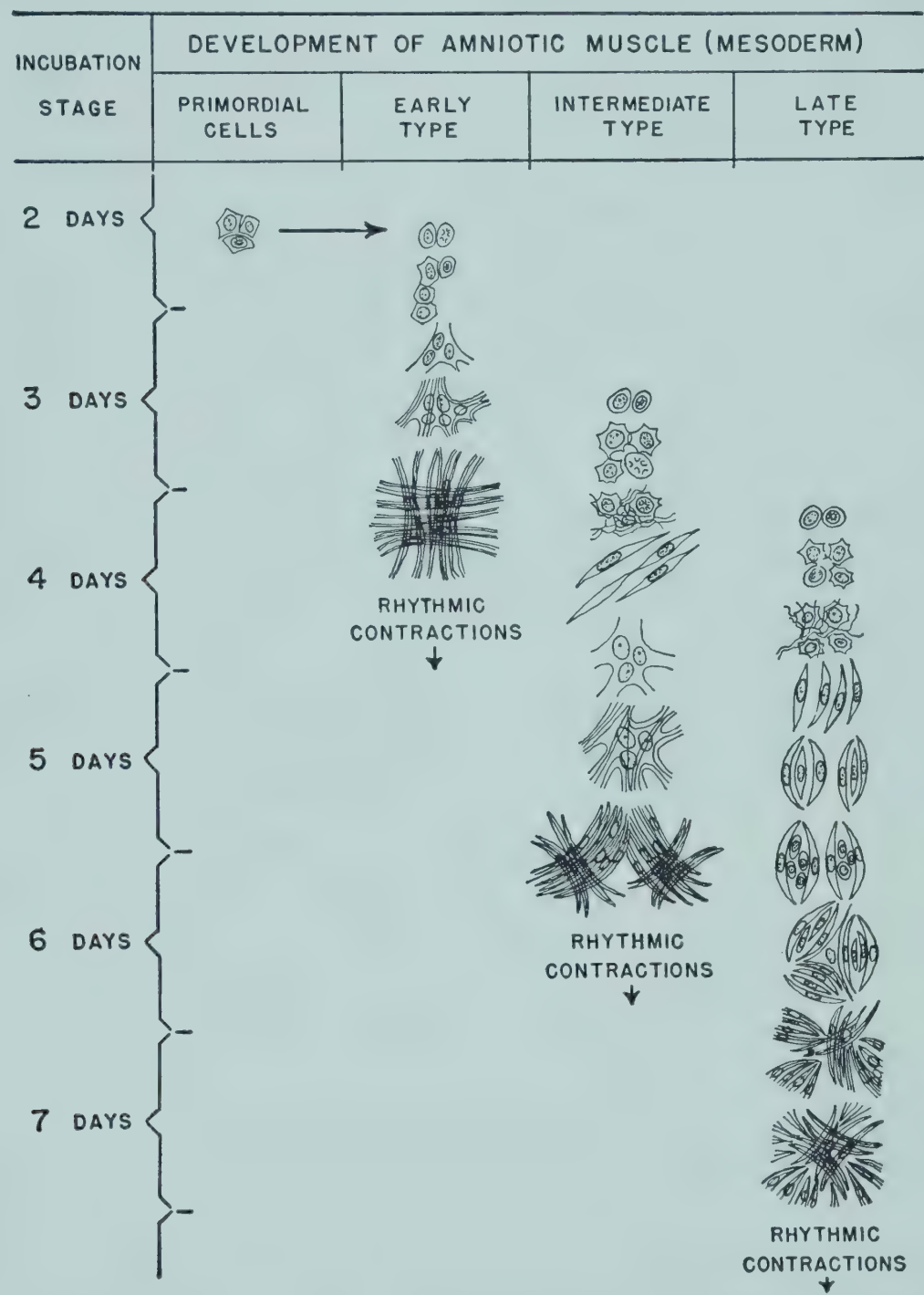


Fig. 405. Representative courses of differentiation of the mesoderm of the chick amnion into active contractile muscle. (Redrawn with modifications after Revoutskaia, 1941, 1944.) All  $\times 150$ .

403). Their radial arrangement around the umbilicus extends halfway to the middorsal line. In the upper zone of the amnion, many of the cells form broad bundles with their long axes parallel, whereas others are grouped radially (Fig. 404-A) about a common center (Fülleborn, 1895; Verzar, 1908; Pierce, 1933; Bautzmann and Schröder, 1953).

The histological differentiation of the amniotic musculature is accompanied by considerable mitotic activity (Verzar, 1908). It is of incidental



interest to note that Lewis and Lewis (1917*b*) determined the duration of the various mitotic phases in the muscle cells of the amnion of a 4-day chick removed to Locke's solution. Their data are given herewith.

Stage of Mitosis	Duration (min.)
Prophase	5-33
Metaphase	2-15
Anaphase	1- 3
Telophase (entire)	3- 5
Early	1- 2.5
Late	1- 4
Reconstruction	4-10

Beginning on the fifth day, the spindle-shaped cells elongate further to form typical smooth muscle elements (cf. Fig. 403; Fig. 405) containing myofibrils (Verzar, 1908). It has been said, however, that fibrils cannot be seen in the living cultured cells (Lewis and Lewis, 1917*a*; Lewis, 1920; Lewis, 1923*a*). By the seventh or eighth day, the individual cells have become about 6 microns wide and 24 to 30 microns long (Pierce, 1933). Remak (1854) stated that they decrease in size after the tenth day because of extensive multiplication.

The musculature of the amnion is characterized by the arrangement of its fiber bundles in peculiar double-layered configurations resembling stars or crosses. The development of the "cross figures" starts at the anterior end of the amnion (Pierce, 1933) around centers composed of a few cells which become irregularly or radially grouped together during the fourth day. According to Verzar (1908), more cells are soon added in parallel alignment around each center to form a bundle. As fibrils from more distant cells join the bundle, they follow a curving course toward it. Eventually, fibrils coming in at an angle from a considerable distance rise up when they reach a bundle and bend around its end in a parabolic arch to meet fibrils coming in at a similar angle on the other side of the bundle (cf. Fig. 404-B). Another parabolic figure forms at the opposite end of the same bundle. Between the two parabolic figures there forms a second bundle of cells whose long axes are at right angles to those of the primary bundle. This second bundle bridges the first. Bautzmann and Schröder (1953), however, gave a somewhat divergent account of the process. They denied that muscle fibers from distant cells direct themselves into the centers of star formation. Instead, the cytoplasm around some of the radially directed nuclei in these centers is sent out in various directions, and the cells become swollen and triangular. Soon the cells arrange themselves in two layers, with the nuclei in one layer transverse to those in the other. Revoutskaia (1939*a*, 1939*b*, 1941) described the early development of star figures in much the same way but added that there is a second, slower type of differentiation,



between the fifth and the seventh day (cf. Fig. 405), which more or less resembles the process observed by Verzar (1908).

A typical cross figure, seen from above, has the appearance of a parallelogram with concave edges and sharply pointed corners (cf. Fig. 404-A and C), although there may be various irregularities of configuration (cf. Fig. 404-D). At a regular distance around a cross figure, new figures may arise, forming circles that are connected with the central complex by means of radial muscle fibers. Growth seems to occur at the periphery of such a field. Sometimes several cross figures lie parallel to each other and are connected with similarly arranged figures by fibers originating at the narrow ends of the parallelograms; in other instances, it is the long sides of the figures which are connected (*Bautzmann and Schröder, 1953*). Revoutskaia (1939*a*, 1939*b*) stated that end-to-end anastomoses and direct ramifications of the cells into each other are prevalent on the fourth and fifth days;



Fig. 406. A schematic representation of the distribution of the cross figures (muscle bundles) and the radial musculature in the amnion of the chick embryo incubated about 9 days. (Redrawn with modifications after *Bautzmann and Schröder, 1953*.)

A, upper surface of the amnion; B, surface turned toward yolk. Both  $\times 5$ .

1, amnion; 2, embryo; 3, cross figures; 4, radial muscles.

that large transverse anastomoses unite groups of cells on the fifth and sixth days; and that after the seventh day connecting strands are given off from the cells at acute angles.

The cross figures are most numerous between the seventh and ninth days. Most of them are found in the dorsal half of the upper side of the amnion; they occupy only the dorsal one third of the side that is turned toward the yolk (Fig. 406). Their concentration per unit area is greatest at the anterior and posterior poles of the membrane. The muscles that radiate from the umbilicus, on the other hand, are longest on the upper side of the sac and are shortest at its anterior end (*Bautzmann and Schröder, 1953*). By the tenth day, the cross figures have grown in size and have decreased in number. Gradually they become so complexly interwoven that they are no longer easily distinguishable. At the end of the twelfth day (cf. Fig. 404-A), they have largely given way to a tangle of muscular bands (*Pierce, 1933*); on the fourteenth day, only one or two cross figures



are found per square millimeter, as compared with ten or twelve on the fifth day (*Clements and Ferguson, 1951*). The mesodermal portion of the 12-day amnion (cf. Fig. 403) is three cell layers thick (*Pierce, 1933*).

The muscle system of the amnion fuses with that of the inner limb of the allantois, beginning in the vicinity of the sero-amniotic connection on the seventh incubation day in both the coot (*Fulica atra*) and the chick (*Fülleborn, 1895; Steinmetz, 1930*). During the second half of the developmental period, the amnioallantoic musculature undergoes involution (*Fülleborn, 1895; Schauinsland, 1906*). By the fifteenth day, the characteristic cross figures and interwoven bands of muscle have entirely disappeared

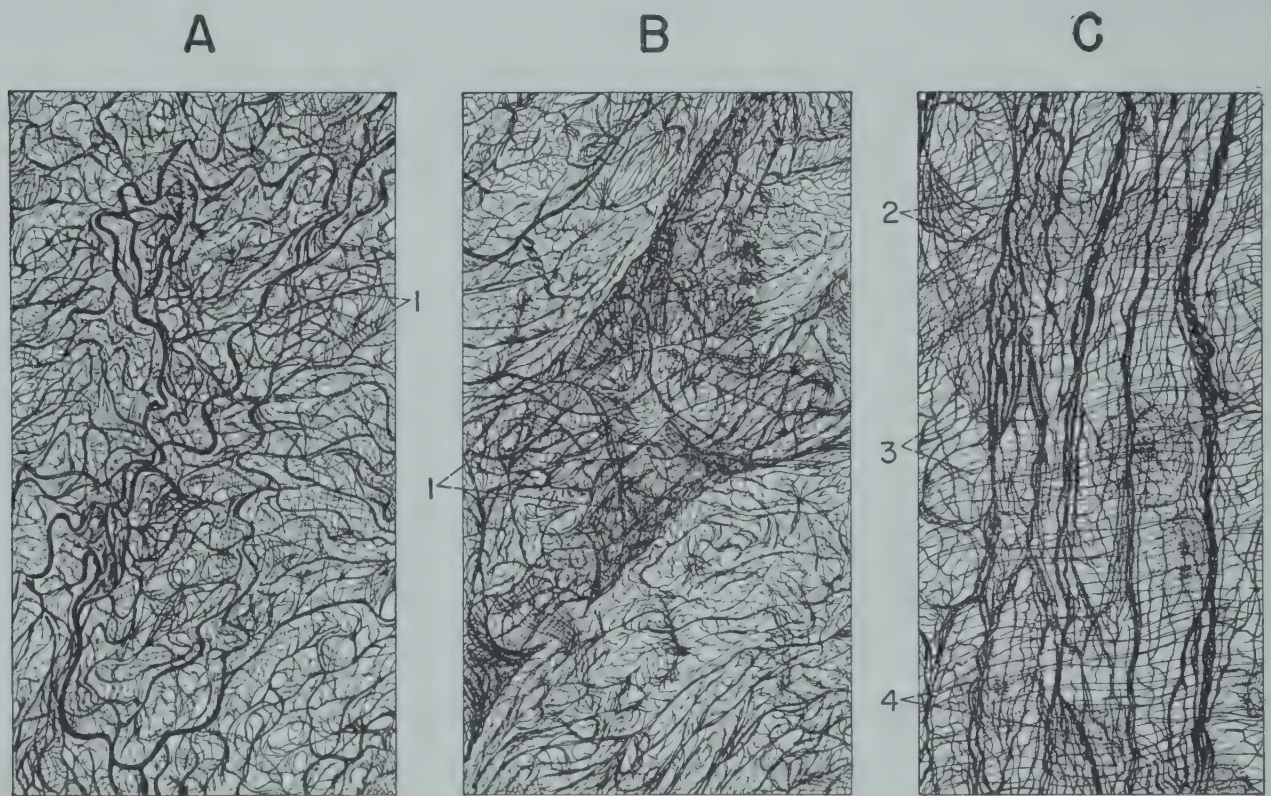


Fig. 407. The supporting fibers in the amnion of the chick embryo, as revealed by the Gomori silver impregnation method. (Redrawn with modifications after Bautzmann and Schröder, 1953.)

A, at 3 days ( $\times 350$ ); B, the supporting fiber network in a cross figure (muscle bundle) at 6 days ( $\times 170$ ); C, at 15 days ( $\times 400$ ).

1, supporting (reticular) fibers; 2, supporting fibers accompanying muscle cells; 3, transverse supporting fibers; 4, epithelial cells.

in the region of amnioallantoic fusion and have been replaced by fine, parallel fibers arranged in two layers. By the seventeenth day, there are numerous lacunae in these fibrous layers (*Bautzmann and Schröder, 1953*).

### *The Supporting Fibers*

A complex system of supporting fibrils (Fig. 407) was demonstrated in the amnion by Bautzmann and Schröder (1953). The investigators stated that the fibrils have no relationship whatsoever to the epithelial cells, as Peterfi (1913) believed, but on the contrary are probably the intercellular product of mesodermal cells (either the muscle cells themselves, or fibrocytes, which, however, could not be identified). On the chick's third



incubation day, appropriate techniques reveal the presence of tangled network of branched and sinuously curving fibrils which vary greatly in thickness, some being relatively massive, others approaching the limits of visibility at high magnification. The network is densest at the points where there are aggregations of myoblastic nuclei. This correspondence between the density of the supporting fibrils and the muscle cell nuclei is even more pronounced after the differentiation of the star and cross figures in the musculature (cf. Fig. 404-B). After the thirteenth day, the system of supporting fibrils has a more regular order and consists of two layers coursing more or less consistently at right angles to each other. The elasticity and toughness of the amniotic membrane is probably related to the presence of these fibrils.

### Contractile Activity of the Amniotic Musculature

Early investigators noted that the chick's amnion exhibits contractile activity on the sixth and seventh days of incubation (*Baer, 1828b; Vulpian, 1857b*). It was established later that the amniotic musculature usually begins to function on the fifth day in the chicken egg (*Preyer, 1879, 1885; Verzar, 1908; Kuo, 1932b; Pierce, 1933*), although it may start to contract in the middle of the fourth day or not until the end of the sixth day. Kuo (1932*b*) observed 458 embryos to determine the relative frequency with which contraction is initiated at various ages; his results are given in the tabulation. In a number of altricial birds, the first contractions occur at a comparable stage of development, that is, after 20 to 25 per cent of the incubation period has passed (*Groebbels, 1933*).

Age of Embryos (hours)	Amnions Initiating Contraction (per cent)
86	0.66
88	6.11
90	2.84
92	6.77
94	17.69
96	13.10
120	43.89
144	8.95

It should be noted, however, that the chick's amnion exhibits contractile activity of a certain type at the 3- and 4-day stage. When the membrane is stretched, it always snaps back to its original size and shape, and excised strips can be stretched three times their length and yet return to their original dimensions immediately after being released. Through their elasticity, the muscles of the amnion give the membrane tonus and keep the sac tense (*Pierce, 1933*). The tonus of the amniotic musculature tends to eliminate variations in endoamniotic pressure (*Remotti, 1932*).



Once amniotic contractions have begun, they rapidly increase to maximal strength and frequency. In the chick, the greatest activity has been observed on the eighth day (Baer, 1828*b*), the ninth day (Kuo, 1932*b*), and the twelfth day (Preyer, 1885) of incubation, or at a stage that is comparable to, or perhaps slightly earlier than, that at which it occurs in several altricial birds (Fig. 408). Various reports indicate that the chick's amnion contracts from eight or ten to twenty times per minute between the fifth and the eighth days (Vulpian, 1857*b*; Preyer, 1885; Clark and Clark, 1914*b*; Pierce, 1933); Kuo (1932*b*) found that the average maximum frequency in 6- to 9-day chicks was eighteen to twenty-five contractions per

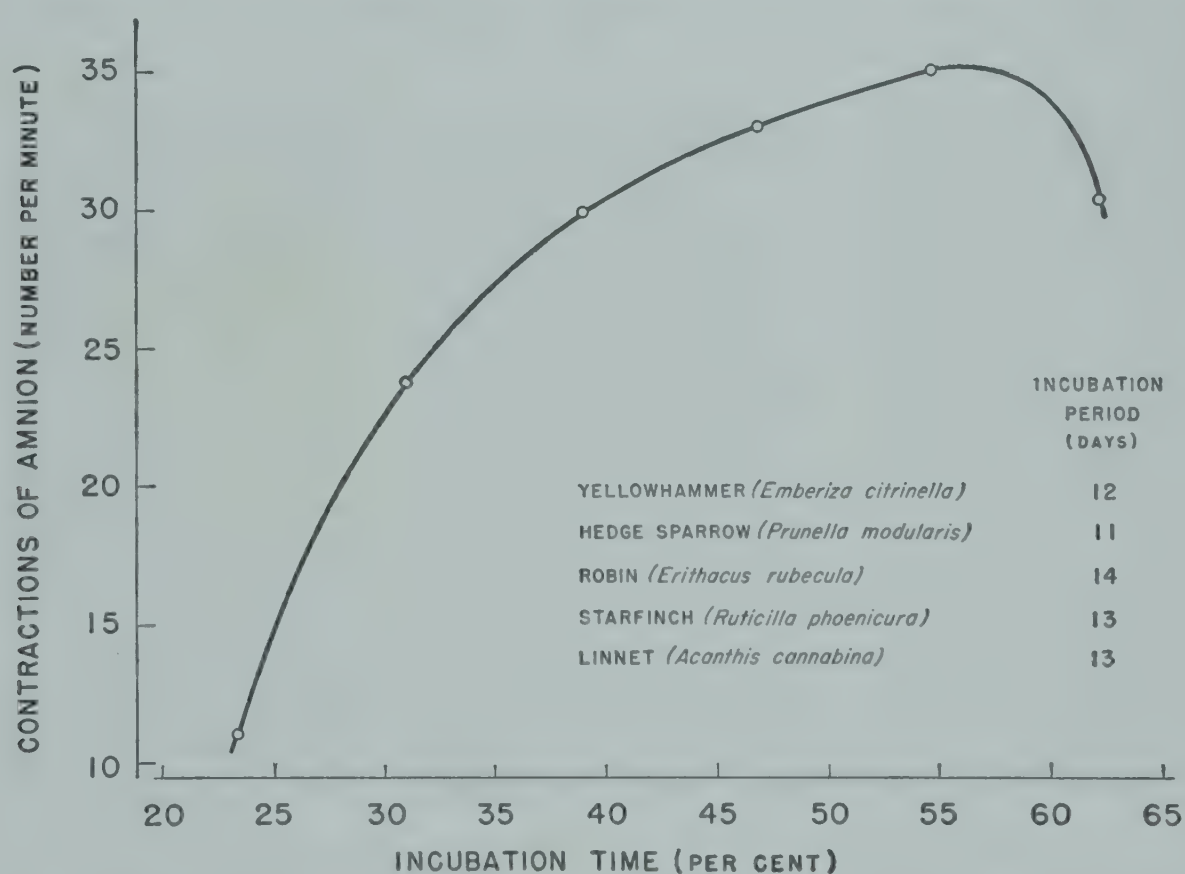


Fig. 408. Changes in amniotic activity occurring in embryo of some altricial birds. (Redrawn with modifications after Groebbe, 1933.)

In the five avian species listed, identical changes in frequency occur at corresponding developmental stages.

minute. It appears likely, therefore, that the amnion of the chick is slightly less active than that of smaller birds (cf. Fig. 408).

The attainment of maximum activity is followed by a brief period of progressive decrease in the vigor, regularity, and rate of the contractions, and finally by their complete cessation. Preyer (1885) remarked that the chick's amnion gradually stopped contracting after the twelfth day; Kuo (1932*b*) found that only a few chick embryos exhibit amniotic activity on the thirteenth and fourteenth days, and none at all on the fifteenth day.

The nature of amniotic activity has been variously described. Baer (1828*b*) observed merely an alternation of contraction from the anterior to the posterior end of the sac, with a consequent pendulous movement of the embryo in a half circle about the umbilicus as a center. Later, this



description was enlarged with the additional information that the contraction proceeds down the amnion in the form of a wave (*Remak, 1854; Verzar, 1908; Clark and Clark, 1914b; Pierce, 1933*) and that the amnion is simultaneously pulled toward the umbilicus by the shortening of the radial muscles (*Verzar, 1908; Pierce, 1933*) in the manner indicated in Fig. 409. Occasionally, a relatively weak contraction wave proceeds from the posterior to the anterior end of the sac (*Clark and Clark, 1914b; Pierce, 1933*). According to *Clark and Clark (1914b)*, some contraction waves extend only halfway down the membrane, and others start at the level of the cervical flexure and proceed cephalad and caudad simultaneously. *Kuo*

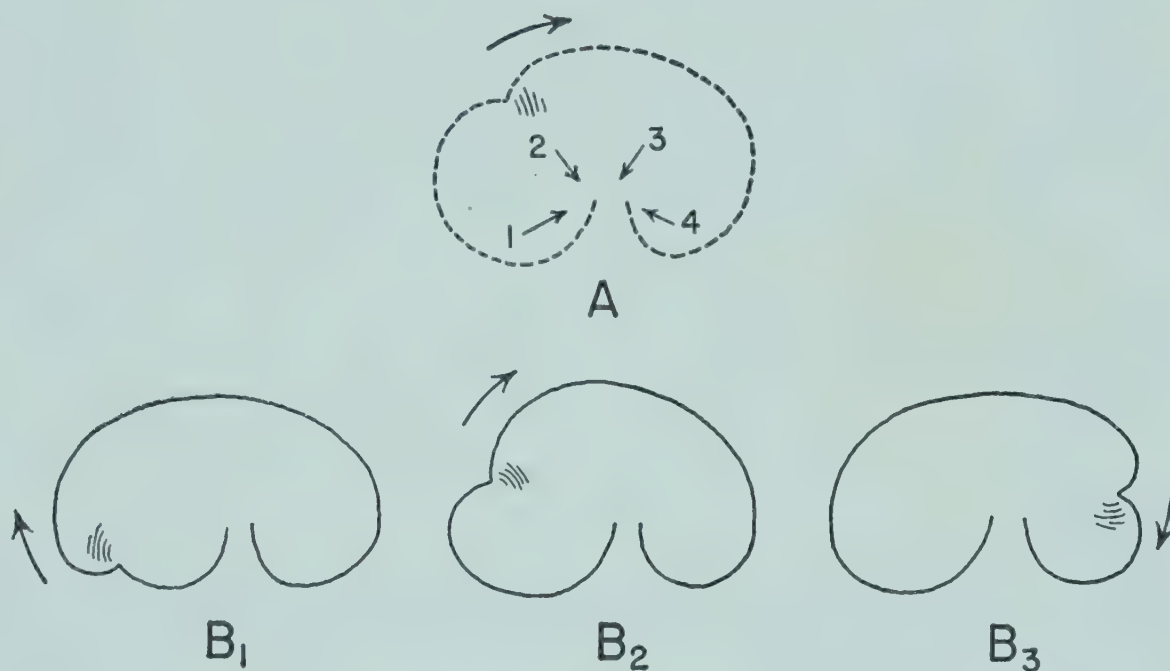


Fig. 409. A diagram of contraction of amnion of the 7- to 8-day chick embryo. (Redrawn with modifications after *Pierce, 1933*.)

A, direction and order of contraction of amniotic cells arranged radially about the umbilicus;  $B_1$ ,  $B_2$ , and  $B_3$ , successive stages in an anteroposterior contraction wave passing over the amnion. Arrows indicate direction of movement of the contracting area.

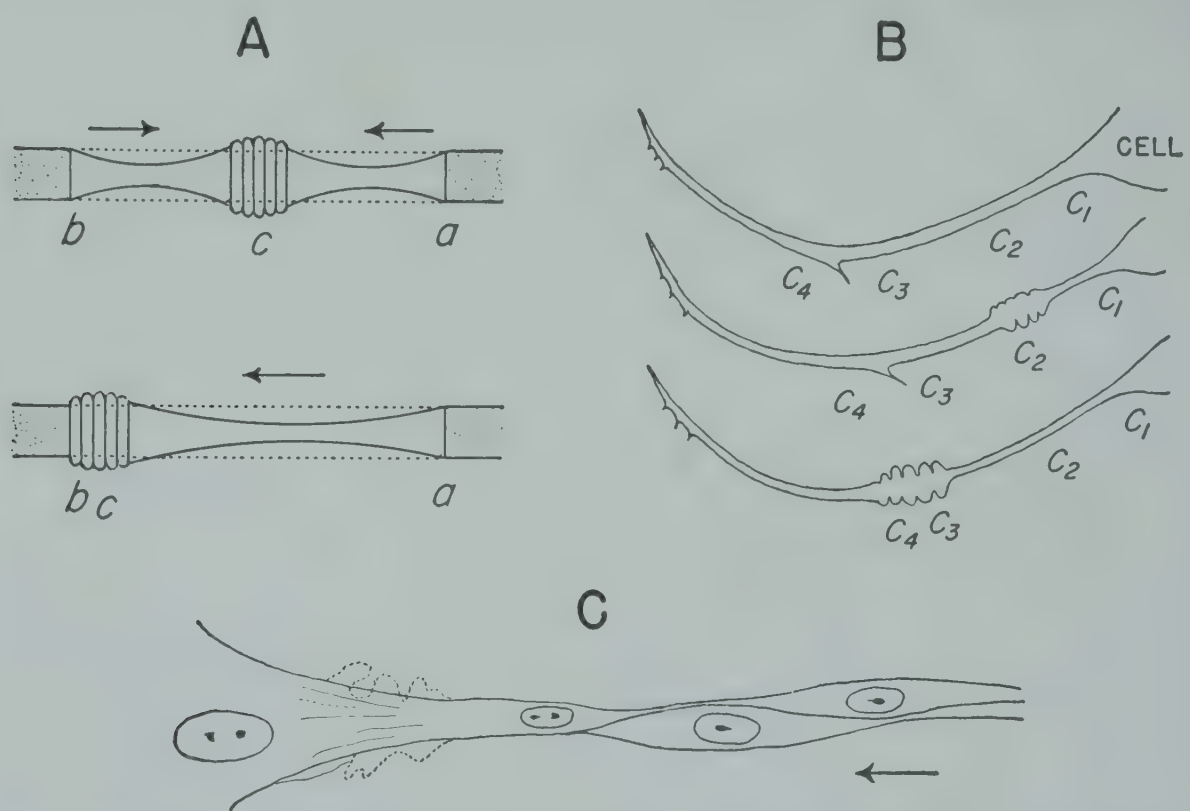
(1932b) stated that contraction may start at any level and proceeds in any direction. In general, however, the anterior end of the side appears to be the pacemaker and initiator of contraction (*Pierce, 1933*).

Because the cross figures of the amniotic musculature are confined to the middorsal region of the sac, where the contractions are most apparent, *Verzar (1908)* believed that the activity of these figures produces the successive contraction waves. *Pierce (1933)*, however, concluded that the radial muscles are chiefly responsible, since the cross figures are arranged too irregularly to give rise to other than local contractions.

Microscopic observation of cultured explants from the chick's amnion indicates that the muscle cells may contract individually or that small bundles of cells may contract together. Contraction consists of a movement of cytoplasm toward a point, the contraction node, where the cytoplasm accumulates and is thrown into folds. As indicated diagrammatically in



Fig. 410-A, the contraction node may be located in the middle or at one end of the active area. Cell processes may contract independently of the remainder of a cell, and the contraction node may appear in several different places in succession (Fig. 410-B). Several cells contracting together as a unit are shown in Fig. 410-C. Under the conditions of tissue culture, each contracting unit has an individual rhythm (*Lewis and Lewis, 1917a; Lewis, 1920*). The phenomenon of contraction in the intact amnion corresponds to that of the cultured tissue, despite the larger number of cells in the active area (*Lewis, 1920*).



**Fig. 410.** Mode of contraction of amniotic smooth muscle cells of the chicken embryo. (Redrawn with modifications A, B, after *Lewis and Lewis, 1917a*; C, *Lewis, 1920*.)

A, a diagram of a contracting cell, wherein *a* and *b* are the regions from which the cytoplasm moves toward *c*, and *c* is the region of shortening and thickening which contains the folds or contraction node; B, a freehand drawing of a cell process with four different regions (*c*<sub>1</sub>, *c*<sub>2</sub>, *c*<sub>3</sub>, and *c*<sub>4</sub>) at which the contraction node successively occurs. The protoplasm always proceeds from the cell body toward the extremity of the process; C, location of a contraction node in a bundle of three cells contracting as a unit from the amnion of a 5-day chick embryo. B and C,  $\times 250$ .

The contractions of the amnion are autonomous and purely myogenic. Repeated search has failed to reveal the presence of nerves or nerve endings in the amniotic musculature of the chick (*Remak, 1854; Verzar, 1908, 1914; Peterfi, 1913; Lewis, 1920; Pierce, 1933; Ferguson, 1940*). Duval (1881) found no neural elements in the amnion of the nightingale (*Luscinia megarhyncha*) and the hedge sparrow (*Prunella modularis*).

The functional independence of amniotic muscles is indicated by the fact that they may continue to contract for as many as 10 days after the natural or experimental death of the embryo (*Wolff, 1932; Remotti,*



1933c). Their inherent contractility is further demonstrated by the ability of excised pieces of amnion to remain active *in vitro* for some time. Remak (1854) was probably the first to observe spontaneous peristaltic movement in bits of tissue cut from the membrane. Strips of amnion perfused in a suitable saline solution may exhibit activity for 20 to 60 minutes after excision. Strips from 12- to 15-day chick amnions contract three or four times per minute, those from 10- to 11-day amnions seven or eight times per minute, and those from younger amnions at a more rapid rate (Ferguson, 1940; Clements and Ferguson, 1951). Figure 411, taken from tracings,

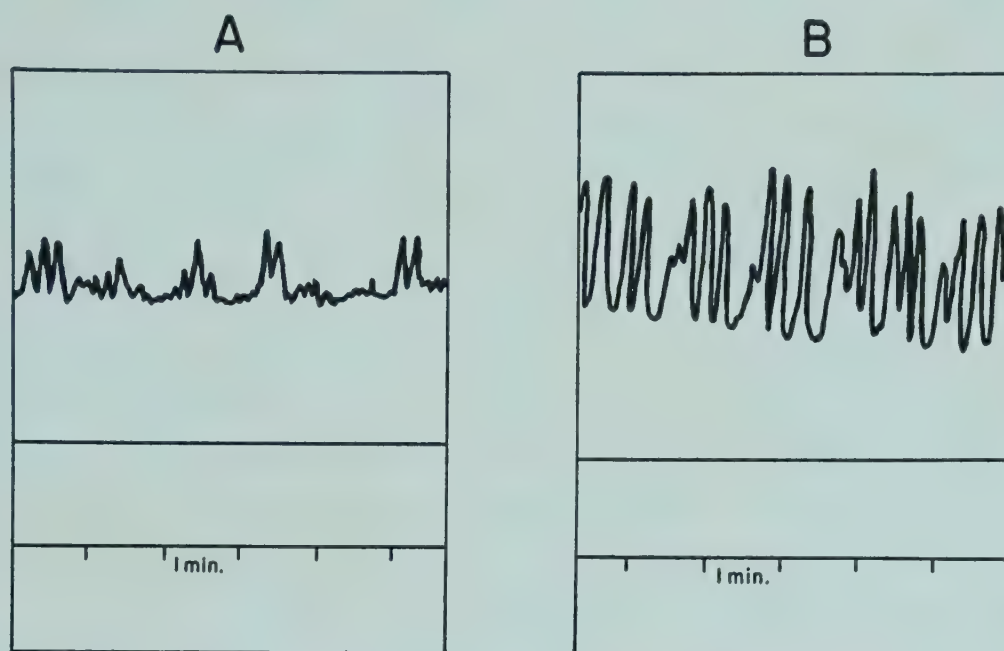


Fig. 411. Variations in rhythmicity and tonus changes in the amnion muscle of the 10- to 13-day chick. (Redrawn with modifications after Ferguson, 1940.)

Two independent observations, in A and in B, of isolated strips of amnion suspended in saline solution.

shows typical rhythm and tonus changes. As previously mentioned, contractile activity is also observed in the muscle cells that migrate from cultured explants of the amnion (Lewis and Lewis, 1917a; Lewis, 1920). The frequency of contraction decreases as the age of the culture increases and has been reported to vary from one to eight per minute in cultures 24 to 48 hours old (Lewis and Lewis, 1917a).

### *Factors Affecting Contractile Activity*

The muscular tissue of the amnion responds to thermal, mechanical, electrical, and chemical stimuli. Its responses are obviously direct, since they occur without the mediation of a neural mechanism. Furthermore, amnions that remain alive and active after the death of the embryo retain their excitability even longer than their spontaneous contractility (Wolff, 1932).

Whether excised or *in situ*, the chick's amnion reacts to a deviation in environmental temperature from the normal incubation level by changing its rate of contraction. Within certain limits, an elevation or depression of the temperature results in an increase or decrease, respectively, in contrac-



tion frequency; beyond these limits, activity ceases entirely (*Lewis and Lewis, 1917a; Pierce, 1933; Ferguson, 1940*). The optimal range of temperature (for excised, perfused strips) is from 38° to 41° C., for the acceleration occurring at 42° C. is not sustained. At 43° C., contractions disappear, but they reappear if the temperature is lowered to 42° C. (*Ferguson, 1940*). Cessation of activity at subnormal temperatures is not necessarily permanent if cooling is of limited duration. Incubation may be interrupted after 5 to 7 days by a 24-hour period of 10° C. without affecting the ability of the amnion to contract for several additional days when the egg is rewarmed, although the embryo is killed (*Wolff, 1932*). A piece of amnion may be held at 23° to 24° C. for 5 minutes (*Ferguson, 1940*) or at -2° C. for 10 minutes (*Ferguson, 1949b*) and still be able to resume contraction when returned to a solution at 41° C. However, contractility is permanently lost by strips held at -3° C. for 10 minutes (*Ferguson, 1949b*). Luyet and Gonzales (1950) observed that small pieces (5 by 10 mm.) of 9- to 17-day amnions, partially dehydrated in 30 per cent glycol, could be held in liquid nitrogen at -195° C. for 1 minute to 1 hour without invariably exhibiting a loss of contractility when returned to 40° C. The activity of a few specimens returned spontaneously within 1 to 3 hours and that of a few others reappeared in response to mechanical stimulation. They remained active only 5 to 15 minutes, however.

The positive response of the amnion to a mechanical stimulus was mentioned by Baer (1828*b*) as well as by many subsequent observers. Touching the amnion with the point of a needle (*Pierce, 1933*), or pinching it (*Ferguson, 1940*), may result in a local contraction, which is often followed by a general contraction. Within 1 or 2 seconds after the touch of the needle, the amnion pulls in from all directions toward the point of contact. The area of local contraction is about 3 or 4 mm. in diameter. When the anterior end of the amnion is stimulated, a contraction of the entire sac almost invariably results, sometimes without being preceded by a local contraction. The first general contraction is followed by several additional contractions. When the stimulus is applied near the middle of the sac, a contraction wave starts at that level, proceeds to the posterior end, and is followed by several complete contractions. Stimulation of the posterior end of the membrane, however, usually evokes no response. It thus appears that the anterior end of the membrane is the pacemaker and that the stimulus proceeds posteriorly, one bundle of muscle fibers stimulating the next; apparently it cannot go anteriorly because the muscles are in the refractory phase (*Pierce, 1933*).

Excised strips of the 11- to 17-day chick amnion can be stimulated by a change in tension. If the muscle strip is quiescent at the time tension is increased or decreased, the response is one or more contractions; an already contracting strip exhibits an augmented amplitude of contraction. There



appears to be a certain tension, within the range of 50 to 100 mg., which is optimum for rhythmic activity in each amnion. Responses to stretch or release are favored by the presence of ethisterone (anhydrohydroxyprogesterone) or an increase of potassium ions in the perfusing solution and are depressed by insufficient aeration (*Ferguson, 1950*).

Using a weak alternating (induced) current, *Pierce (1933)* applied momentary shocks to the intact amnion of the chick and found that a single shock was without effect but that two or three shocks in rapid succession were followed by several complete contractions. Stimulation of the anterior half of the amnion produced more active responses than stimulation of the posterior half. Very strong shocks easily induced contractions. *Duval (1881)* found amnions of the nightingale (*Luscinia megarhyncha*) and the hedge sparrow (*Prunella modularis*) to be responsive to electrical stimuli.

The contraction frequency of excised strips of amnion can be modified by changing the concentration of certain ions in the perfusion medium. An increase in either the calcium or the potassium ion accelerates the rhythm, and a decrease in potassium (from 5.1 m.eq./liter to 3.18 m.eq./liter) may lead to permanent paralysis. The addition of magnesium to a magnesium-free solution causes a decrease in contraction rate when the ionic concentration reaches 1.04 m.eq./liter. An increase in acid phosphate augments muscular activity but an increase in basic phosphate has no definite effect (*Ferguson, 1940*).

TABLE 23

Effects of Drugs and Chemicals on the Muscular Activity of the  
Amnion of the Chick, *Gallus gallus* \*

Substances Used	Stimulation (+) or Depression (—)
Ergotamine tartrate	++
Acetylcholine	++
Strophanthin	++
Muscarine	++
Physostigmine	+
Pilocarpine	+
Hypophysin	+
Choline	+
Caffeine	±
Pentamethylene tetrazole	—
Dilaudide	—
Tincture of opium	—
Epinephrine	—
Papaverine	—

\* After *Baur (1928)*.



According to Baur (1928), who tested the effect of various substances on the activity of strips excised from chick and goose (*Anser anser*) amnions, the most powerful stimulant is barium chloride. It reinforces the action of all other stimulants and counteracts the effect of all depressants. Pierce (1933) found that copper chloride (1/10,000) was the most reliable stimulant among a number of reagents (not specified).

Because of its lack of nerves, the amnion has been used in experiments designed to clarify the question of whether certain drugs act upon nerve endings or upon receptor substances (or other mechanisms) of the muscle cell. Some of the results obtained by Baur (1928) are summarized in

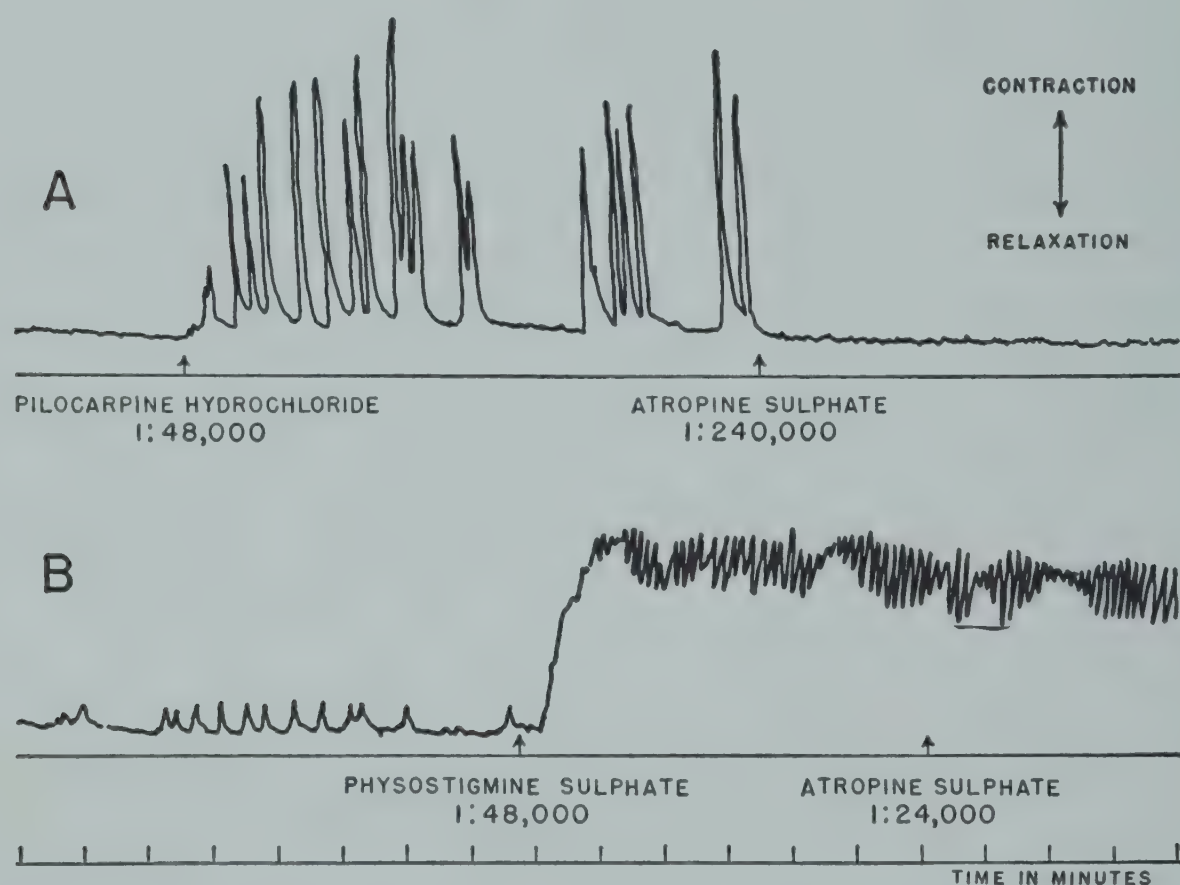


Fig. 412. Response of excised amniotic muscle strips of the 12-day chick embryos to drugs. (Redrawn with modifications after Ferguson, 1949a.)

A, response of a quiescent muscle to pilocarpine hydrochloride followed by inhibition caused by atropine sulphate; B, effect of physostigmine sulphate followed by atropine sulphate.

Table 23. Many of his experiments were repeated by Ferguson (1940, 1949a), who, like Baur, found that acetylcholine, pilocarpine, and physostigmine have a stimulating effect (Fig. 412-A) and that adrenaline is inhibitory for a period and to an extent that is proportional to its concentration. Ferguson (1949a) observed, in addition, that adrenaline may be stimulating to amnions previously treated with progesterone. To the list of stimulants in Table 23 may be added the following substances: digalen, ephedrine hydrochloride, tyramine hydrochloride (Ferguson, 1940), and histamine (Ferguson, 1949a). Benadryl, a histamine antagonist, is a depressant (Ferguson, 1949a). Strychnine has no effect (Langley, 1905).



A few substances vary in their action according to their concentration. Chloretone as a 2 per cent solution paralyzes the (intact) amnion (*Pierce, 1933*) but has no effect when very dilute (*Ferguson, 1940*). Although Langley (*1905*) found nicotine to be a stimulant, Baur (*1928*) observed that it is inhibitory in strong concentrations ( $1/50,000$ ) and causes acceleration in weak concentrations ( $1/500,000$ ). Atropine sulfate varies in its activity in the same way and over roughly the same range of concentration (*Baur, 1928; Ferguson, 1940, 1949a*). It antagonizes the increase in contraction rate and amplitude produced by pilocarpine (*Ferguson, 1949a*), acetylcholine (*Baur, 1928; Ferguson, 1940*), ergotamine tartrate, muscarine, and (when used in a high concentration) barium chloride (*Baur, 1928*), but has little effect after physostigmine (Fig. 412-B). Cocaine, found by Baur (*1928*) to be ineffective, was reported by Ferguson (*1940*) to cause a decisive increase in amniotic activity at a concentration of  $1/480,000$  and to produce paralysis in concentrations stronger than  $1/60,000$ . Its stimulating effect is opposed by adrenaline; it, in turn, opposes stimulation by ephedrine and tyramine (*Ferguson, 1940*). There is some evidence that curarine and d-tubocurarine hydrochloride are either inactive (*Baur, 1928*) or slightly stimulating in solutions weaker than  $1/10,000$  (*Ferguson, 1952*); strong solutions ( $1/4000$ – $1/500$ ) of the latter compound are depressing and decrease or abolish the response to acetylcholine (*Ferguson, 1951, 1952*).

### The Resistant Properties of the Amnion

Studies of the resistance of the amnion to various forces seem to indicate that the normal course of amniotic development represents a state of equilibrium between the intrinsic potential autonomy of the membrane and the correlative influence of the embryo (*Remotti, 1933c*). Near the end of incubation, the amnion suffers a sudden and total loss of autonomy coincident with, or immediately prior to, its structural involution.

The semi-independence of the amnion from the embryo is revealed by the fact that the development of the membrane may proceed in a normal fashion and at an approximately normal rate for several days after the death of the embryo, provided, of course, that incubation is continued. Wolff (*1932*) and Remotti (*1933c*) observed that the spontaneous or experimental death of the 1- to 3-day embryo is followed within little more than the usual period of time by closure of the amniotic sac, the appearance of amniotic fluid, the differentiation of muscles, and the onset of contractile activity. The amnion may live as much as 11 or 12 days longer than the embryo (*Wolff, 1932*), but its period of survival is inversely proportional to the age of the embryo at the time of death (*Remotti, 1933c*).

The superior viability of the amnion at  $38^{\circ}$  C., as compared to strictly embryonic tissues, was demonstrated in another way by Wilburg (*1937*). He continued the incubation of exsanguinated 9-day chick embryos and



determined the survival time of various tissues by explanting them to culture media at intervals and observing their ability to grow. The amnion remained alive for 221 hours; skin was the only other tissue that survived the embryo by more than 6 hours.

The amnion is less susceptible than the body tissues to the ill effects of temperatures above the normal incubation level. When exposed *in situ* to a temperature of 45.5° C., the amnion (amnioallantois) of the 10-day chick retains the ability to regenerate in culture for 11 to 15 hours, or three to four times as long as skin, the most heat-resistant embryonic tissue (Szarski, 1948). At 48° C., cultures of the 11-day amnion show injury when heated for 30 to 40 minutes, and all the newly grown cells are killed in 40 to 55 minutes; yet both epithelial and mesenchymal elements may grow out from the center of the explant some time after exposure to this temperature for 80 to 125 minutes. When compared with other tissues, it appears that the explanted amnion is considerably more resistant to overheating at 48° C. than the intestine and spleen but only slightly more resistant than the heart and skin (Szarski, 1946).

At temperatures below that of normal incubation, the amnion again exhibits a pronounced capacity for survival. Wolff (1932) was able to study the development of the amnion in the absence of the embryo because of the fact that the latter is killed when the egg is kept at 0 to 1° C. for 5 days, whereas the amnion continues to live. The persistence or return of contractile activity during or after temporary exposure to low temperatures also reveals the resistance of the amnion to chilling. The actual survival time of the 6- to 12-day chick amnion and other tissues at various sub-normal temperatures was determined by Bucciante (1931), who exposed the intact egg to cold and removed explants to culture media at intervals. His data for the amnion are given herewith. Comparison of these figures with those for other tissues indicates that the resistance of the amnion to

Temp. (° C.)	Survival Time of:	
	Amniotic Epithelium (hours)	Amniotic Muscle (hours)
15 to 20	168	120
0	408	240
-4 to -6	28	28
-10	18	18
-14	11	11

cold is less than that of the skin, cornea, meninges, and skeletal muscles; also, the superiority of the amnion to certain other tissues seems to become progressively less as the temperature decreases.

The resistance of the chick's amnion to mechanical injury is greatest in an area that occupies the middle of the superior surface of the membrane



(Remotti, 1933a). Mechanical resistance decreases in every direction from here to the umbilicus. As incubation proceeds, the resistance of the entire amnion increases, but at a different rate in each region. The most rapid increase occurs in the highly resistant middorsal area, where a maximum is attained on the twelfth day; thereafter, the resistance of this sector remains constant until the seventeenth day, and then rapidly declines (Fig. 413-D). In the rest of the amnion, maximum resistance is not reached until the seventeenth day and is followed immediately by a sharp decrease (cf. Fig. 413). Resistance to autolysis varies temporally and spatially in exactly the same manner as resistance to mechanical injury (Remotti, 1933a).

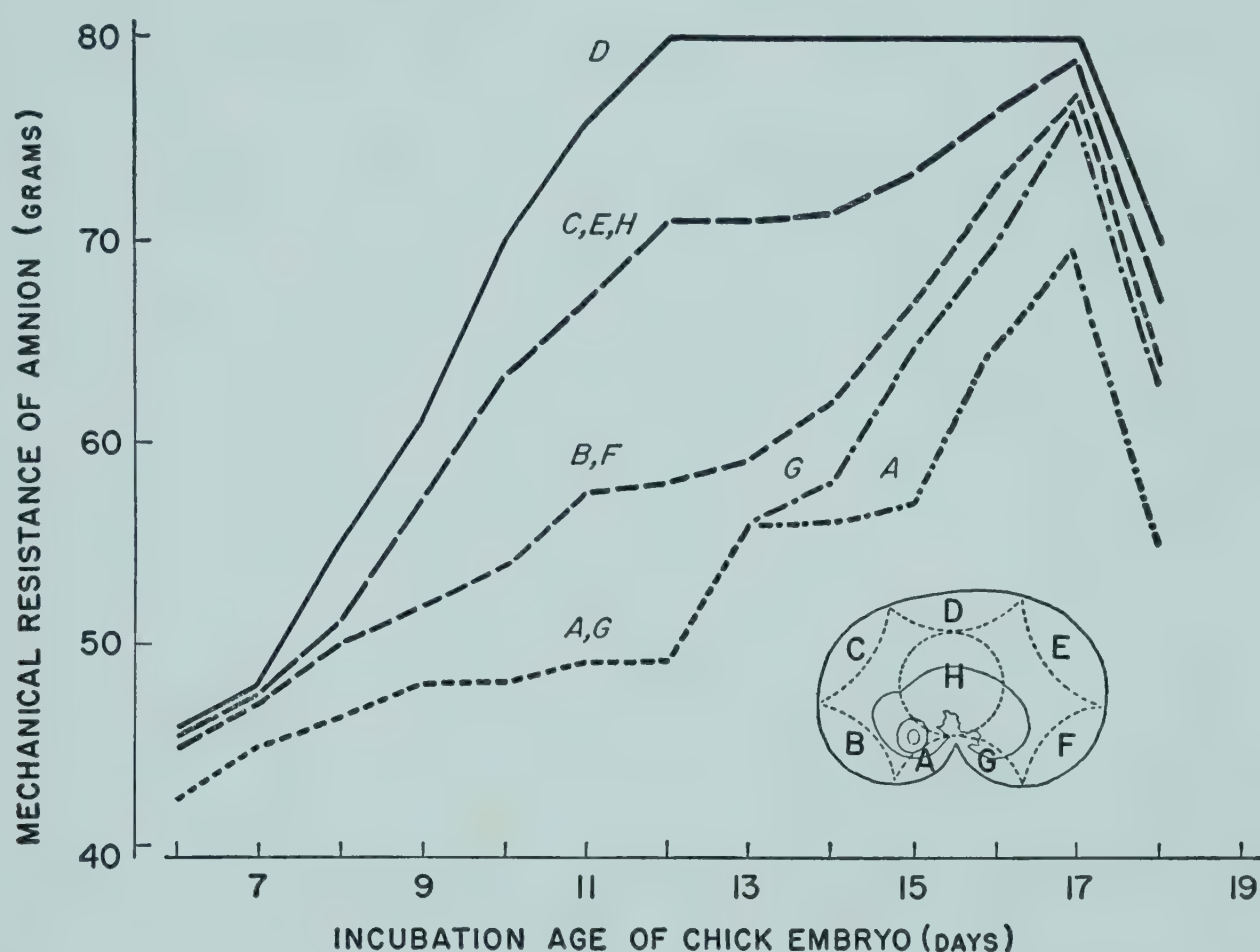


Fig. 413. Curves showing the changes in the mechanical resistance of various regions of the developing chick's amnion. (Redrawn with modifications after Remotti, 1933a.) Resistance is expressed in terms of the weight causing rupture of the sac.

### The Amniotic Fluid—Formation and Absorption

The amniotic sac is closely applied to the embryo in the beginning but is soon carried away from the body as thin, clear fluid accumulates in the amniotic cavity in increasing amounts. The origin of this fluid, although not definitely established, seems to be extraembryonic, since amnions that develop in the absence of the embryo are nevertheless fluid-filled (Wolff, 1932). The fluid, if not a secretion of the amniotic wall, may be a transudate from the blood vessels of the area pellucida (Giacomini, 1930a, 1930b). On the chick's fifth day of development, the amount of liquid within the amnion is sufficient to keep the sac well away from the embryo



(Pierce, 1933). The volume of fluid continues to increase until it attains a maximum of 3 to 4 cc. on or about the thirteenth day of incubation (Kamei, 1927; Romanoff and Hayward, 1943; Kugler, 1945). This quantity represents 8 to 9 per cent of the original weight of the egg (Romanoff and Hayward, 1943).

From its maximum, the amount of fluid usually declines slightly until the fifteenth day, then rises again, and finally decreases to zero rather rapidly during the last few days of incubation. As may be seen in Fig. 414, changes in the volume of amniotic fluid during incubation follow much the same course in the chick (*Gallus gallus*), the turkey (*Meleagris gallopavo*), the ring-necked pheasant (*Phasianus colchicus*), the bobwhite quail (*Colinus virginianus*), and the Pekin duck (*Anas platyrhynchos*).

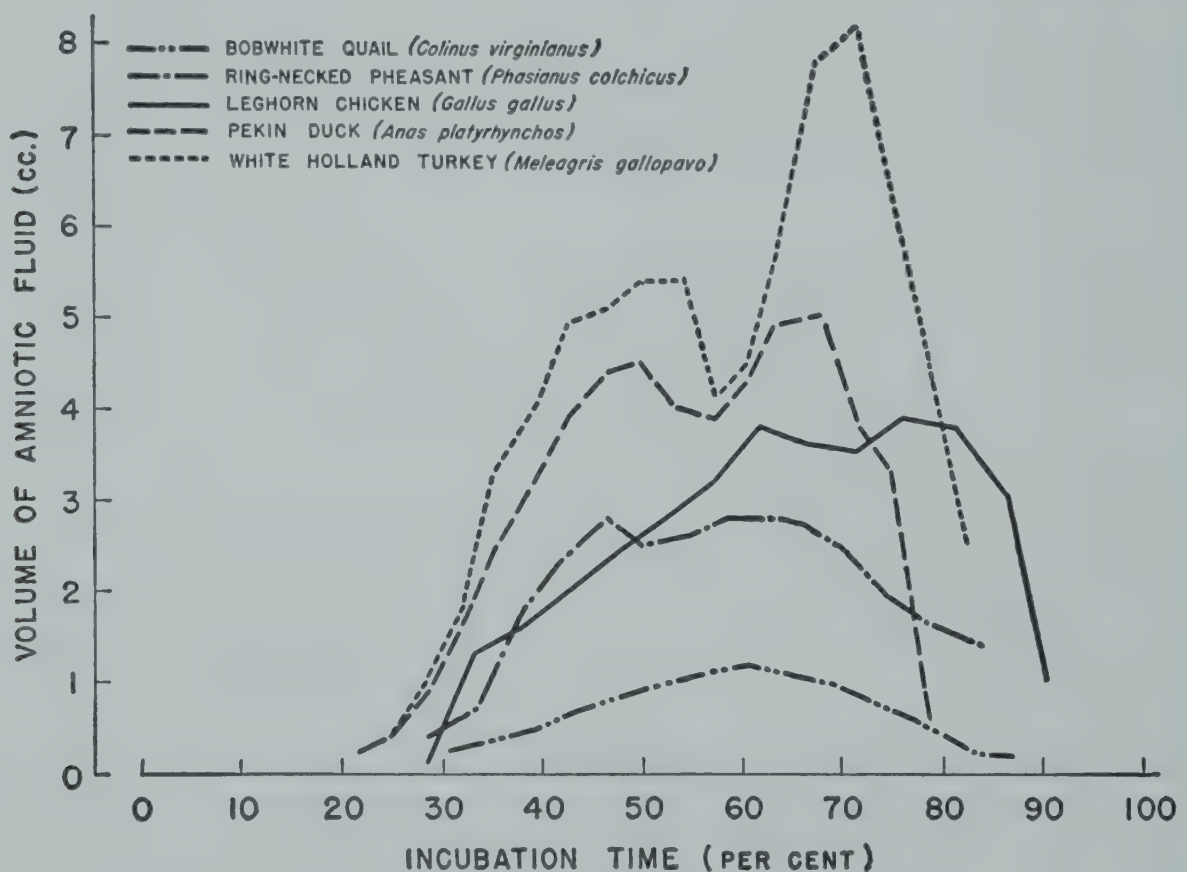


Fig. 414. Changes in volume of amniotic fluid during incubation of several species of birds. (After Romanoff and Hayward, 1943.)

The increase in the fluid contents of the amniotic sac after the chick's tenth day must be attributed chiefly to the influx of albumen through the ruptured sero-amniotic connection. Conditions that retard or accelerate the perforation of the sero-amniotic plate therefore affect the fluctuations in the volume of amniotic fluid. The volume of fluid may be less than normal in unturned eggs (Randles and Romanoff, 1949, 1950). Incubation at a subnormal temperature causes a delay of a day or two in the accumulation of the maximum amount of fluid. When the incubation temperature is too high, the intermediate decrease in the quantity of fluid begins prematurely and is followed, between the thirteenth and sixteenth days, by a precipitous rise to a volume of about 6 cc. (Ogorodniy and Penionschkevitsch, 1939; Romanoff and Hayward, 1943).



The final phase of decrease is thought to be due almost entirely to the ingestion of the fluid by the embryo. Evidence that the embryo actually swallows the liquid contents of the amnion is provided by the appearance of various substances in the digestive tract after their injection into the amniotic sac. The observations of Vrbitch (1924), who injected lycopodium powder into the sac, indicate that the chick embryo may begin to swallow the fluid on the tenth or twelfth day and is certainly ingesting it on the fourteenth and sixteenth days. Wislocki (1921) injected trypan blue into the amniotic cavity of the 11-day chick and 2 days later found the dye not only in the amniotic fluid and the epithelial cells lining the amnion but also in the stomach, intestines, trachea, and primary bronchi. Similar results were obtained by Taylor and Saenz (1949), who used Evans' blue, suspended charcoal, and suspended radioactive chromic phosphate; furthermore, the latter investigators observed that double  $P^{32}$  (in the form of dibasic sodium phosphate) introduced into the amniotic sac is soon detectable in the blood and body of the embryo as well as in the allantoic fluid. Trypan blue, trypan red, methylene blue, fluorescein, and vegetable dyes appear in the amniotic fluid and the digestive tract if injected directly into the albumen or if injected into the air chamber of the egg before closure of the albumen sac and in sufficient quantity to diffuse into the albumen (Zaretsky, 1910; Hanan, 1927; Romanoff, 1951a).

### THE ALLANTOIS

The allantois is the last of the extraembryonic membranes to appear. It is a stalked sac which grows out into the extraembryonic coelom under the guise of an appendage of the hind-gut. Eventually, it encloses the entire contents of the egg in a double-walled, fluid-filled envelope.

The allantoic sac performs two extremely important functions. It is the embryonic respiratory organ, and it is the repository for the waste products of the kidneys. Only its external wall participates in respiration; its internal wall has a more or less mechanical significance. The outer and inner walls, or limbs, are different structurally as well as functionally. The outer limb fuses with the chorion and, lying directly beneath the shell membranes, lines the internal surface of the eggshell. It is thicker than the inner limb, of greater superficial extent, and much better vascularized. Its large area, its proximity to the external atmosphere, and its exceedingly rich capillary network combine to make it extremely well adapted to its respiratory function. Certain characteristic structures differentiate in a portion of the outer limb which, as the so-called albumen sac, surrounds and segregates the albumen and assists in the absorption of the latter. The inner allantoic limb, which fuses with the amnion, contains smooth muscle tissue and the proximal portions of the blood vessels which terminate in the outer limb.



### General Developmental History of the Allantois

The allantois starts its development as an autonomous structure, but the direction of its growth and its final configuration are subject to the modifying influence of various factors external to itself. Among these are the position of the embryo, the spatial relationships between the allantois and the rest of the egg's contents, the distending influence of the urinary excretions flowing into the allantoic cavity, the difference in the growth rates of the allantoic blood vessels and walls, and the impediment offered to allantoic expansion by the sero-amniotic connection.

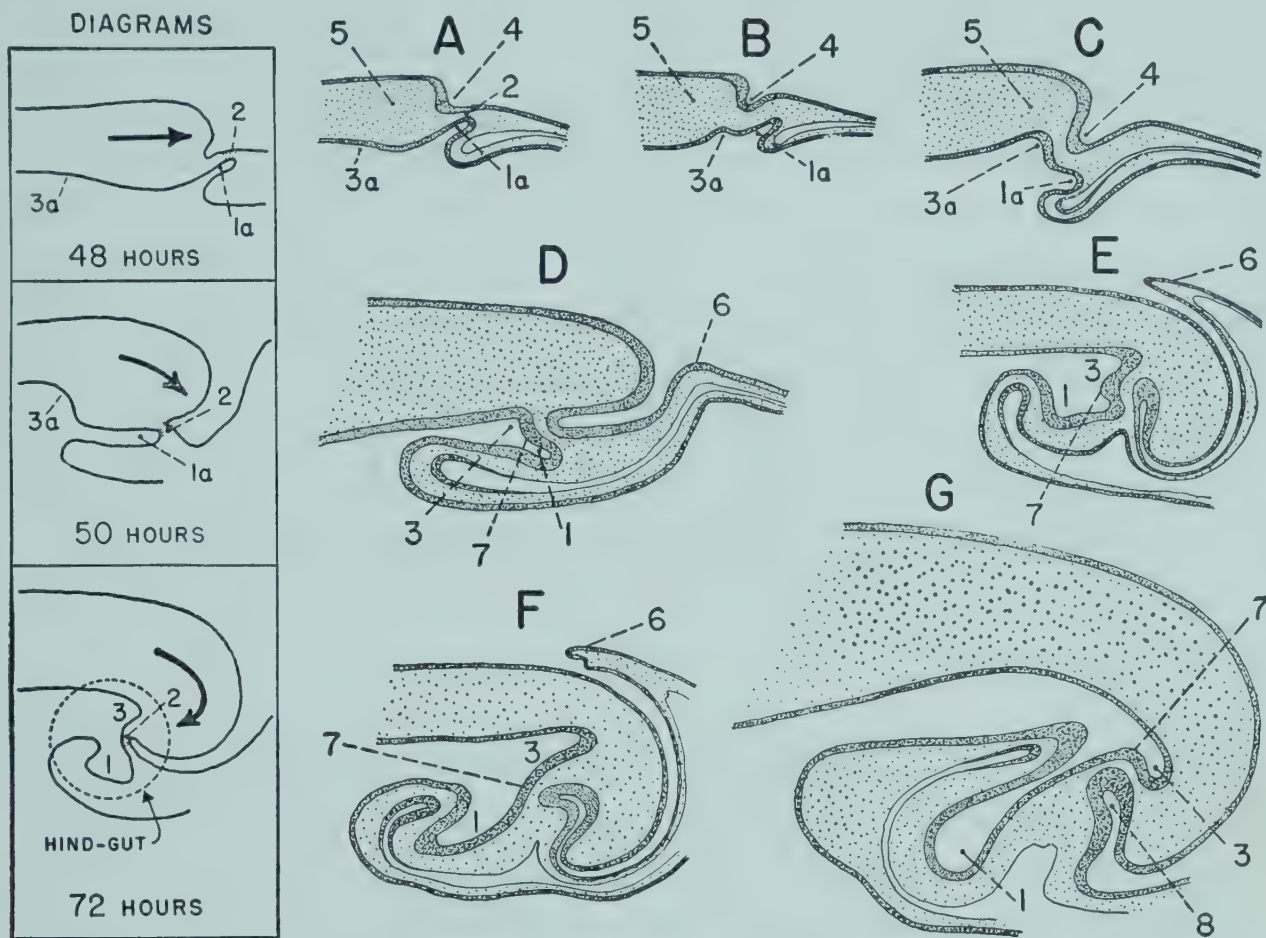
#### *The Initial Development of the Allantois*

The allantois grows out into the extraembryonic coelom as a diverticulum of the hind-gut (*Pander, 1817; Baer, 1828b; Valentin, 1835, pp. 427, 548*). The hind-gut, however, arises slightly later than the allantois, hence the allantois is not derived from it. In the chick embryo, the earliest rudiment of the allantois appears near the end of the second incubation day, between the 20- and 28-somite stages (*Duval, 1877, 1889; Zwillling, 1946*). The structure has usually been described as a dorsocaudally directed evagination of endoderm located below and behind the incipient tail bud. It would perhaps be more accurate to consider this endodermal pouch the result of an anteriorly directed bend in the extraembryonic splanchnopleure posterior to the embryo. The anterior or upper wall of the inverted pocket is formed of embryonic endoderm and its posterior or lower wall of extraembryonic endoderm; its apex, which is very close to the ectoderm, represents the anal plate (Fig. 415-A). This initial stage in the development of the allantois was not seen by some nineteenth century investigators (*Pander, 1817; Baer, 1828b; Valentin, 1835, pp. 427, 548*); others believed that the allantoic anlage was at first double or solid, or both, and later became single and hollow (*Reichert, 1840, p. 186; Remak, 1855, p. 57; Bornhaupt, 1867; Goette, 1867; Schauinsland, 1891; Ravn, 1898*). The concept of a solid allantoic primordium perhaps arose from the circumstance that the mesoderm contiguous to the endodermal pocket is somewhat increased in thickness (*Gasser, 1874*). This mesodermal thickening lies in the zone of transition from the extraembryonic splanchnopleure to the extraembryonic somatopleure (*Ravn, 1898*).

As the tail bud enlarges, it becomes delimited posteriorly by the tail fold (Fig. 415-A, B, and C), an indentation in the somatopleure continuous with the lateral limiting sulci. The tail fold, it should be noted, forms anterior to the anal plate (which, as already mentioned, is at the apex of the allantoic primordium). A second endodermal pocket now begins to form, anterior to the first one and anterior to the tail bud also (cf. Fig. 415-B). This second pocket, which is composed entirely of embryonic endoderm,



represents the caudal or post-anal portion of the hind-gut (*His*, 1868, *p.* 159; *Dobrynin*, 1871; *Gasser*, 1874; *Olivetti*, 1874; *Duval*, 1877; *Gruenwald*, 1941b; *Zwilling*, 1946). The separate and distinct origins of hind-gut and allantois are indicated by the observation that excision of the first endodermal pocket interferes with the development of the allantois but does not prevent normal formation of the hind-gut (*Zwilling*, 1946).



**Fig. 415.** Longitudinal sections of the caudal portion of the chick embryo showing the successive stages in the early development of the allantois. (Redrawn with modifications after *Duval*, 1877.)

*Insert:* schematic drawings (by the author). The arrows indicate the direction in which the caudal portion of the embryo grows. Within the broken-line circle is the hind-gut which, at 72 hours of incubation, contains the caudal intestine, the allantoic diverticulum, and the cloacal membrane.

A, at 48 hours; B, at 49 hours; C, at 50 hours; D, at 52 hours; E, at the middle of the third day (about 60 hours); F, at the end of the third day (about 70 hours); G, at the beginning of the fourth day (about 80 hours). All  $\times 10$ .

1a, allantoic primordium; 1, allantois; 2, cloacal membrane; 3a, primordium of tail gut or caudal intestine; 3, tail gut or caudal intestine; 4, embryonic tail fold; 5, tail bud; 6, tail fold of amnion; 7, cloacal bulge; 8, proctodaeum.

The hind-gut pocket soon grows more pronounced and becomes separated from the rudimentary allantois by a mound of tissue bulging anteriorly (cf. Fig. 415-C). As the yolk stalk constricts, the hind-gut is provided with a floor, and the allantoic pocket is incorporated into the hind-gut as a diverticulum (Fig. 415-D). The tail bud, by growing caudad and downward, causes the axis of the allantoic rudiment to change direction, first from dorsocaudal to horizontal, then to ventrocaudal, until, by the 60-hour stage,



it is dorsoventral (Fig. 415-B, C, D, and E). By this time, the same process of rotation has brought the hind-gut pocket dorsal to the allantois rather than anterior to it. Eventually, by the 30-somite (Gruenwald, 1941b) or 70-hour (Duval, 1877) stage, the original relationship of the two endodermal pockets is completely reversed (Fig. 415-F), for the allantois is now anterior to the hind-gut; also, its axis has undergone further rotation and is directed ventrocraniad. The anal plate, originally at the blind dorsal end of the allantoic primordium, has shifted to the caudal border of the allantois; and the endoderm originally forming the anterior wall of the allantoic primordium has been incorporated into the floor of the hind-gut and will probably become part of the cloaca (Zwilling, 1946). It is either at this stage or early in the chick's fourth day of incubation (Fig. 415-G) that the allantois becomes clearly visible as a small pediculated sac projecting from the body into the extraembryonic coelom. A stalked allantois is present in the 38-somite Emperor penguin (*Aptenodytes forsteri*) embryo (Glenister, 1954) and is visible in the turkey (*Meleagris gallopavo*) at the end of the fourth day (Phillips and Williams, 1944). In the coot (*Fulica atra*), the entire chronology of allantoic development is much the same as in the chick (Steinmetz, 1930), whose incubation period is only 1 or 2 days shorter.

Because of its mode of origin, the allantoic sac of the 4-day chick embryo is composed of an endodermal lining only one or two cells thick, covered externally by a thicker layer of mesenchyme; the latter is already vascularized. The endodermal lining is continuous with the endoderm of the intestine, and the mesenchymal component is continuous posteriorly with the mesoderm of the amnion, anteriorly with that of the ventral body wall (cf. Fig. 415-G).

### *The Growth of the Allantois*

During the course of the chick's fourth incubation day, the stalked allantois grows out on the right side of the embryo (which now lies on its left side) into a space bounded above by the chorion, below by the yolk sac, and on the left by the amnion (Dareste, 1863). The diameter of the allantois increases from 1.0 or 1.5 mm. at the beginning of the fourth day (Fig. 416-A) to 3.0 or 4.0 mm. by its end (Boyden, 1924). It appears that the growth of the allantois is independent of external factors until the end of the third day and is thereafter the result of mechanical distention by the mesonephric excretions, as Dutrochet (1837) assumed. These excretions begin to reach the allantois through the Wolffian ducts during the fourth day. Experimental interruption of the Wolffian ducts at the beginning of the third day prevents urine from collecting in the allantois, and the sac remains very small (Boyden, 1924). As Düsing (1884) showed, the allantois does not grow most rapidly in the direction of the air cell at the



blunt end of the egg; thus it fails to exhibit the "air tropism" attributed to it by early investigators who varnished parts of the eggshell and claimed that the allantois was attracted toward the unvarnished areas (*Baudrimont and St.-Ange, 1847; Dareste, 1855*).

As the allantois continues to enlarge, it spreads toward either pole of the egg (*Dareste, 1863*). By the end of the fifth day of incubation, the allantois of the chicken or the coot (*Fulica atra*) is about the same size as the embryo (*Hirota, 1894; Steinmetz, 1930*). This stage of allantoic development is attained on the turkey's (*Meleagris gallopavo*) sixth incubation day (*Phillips and Williams, 1944*). By the end of the chick's seventh

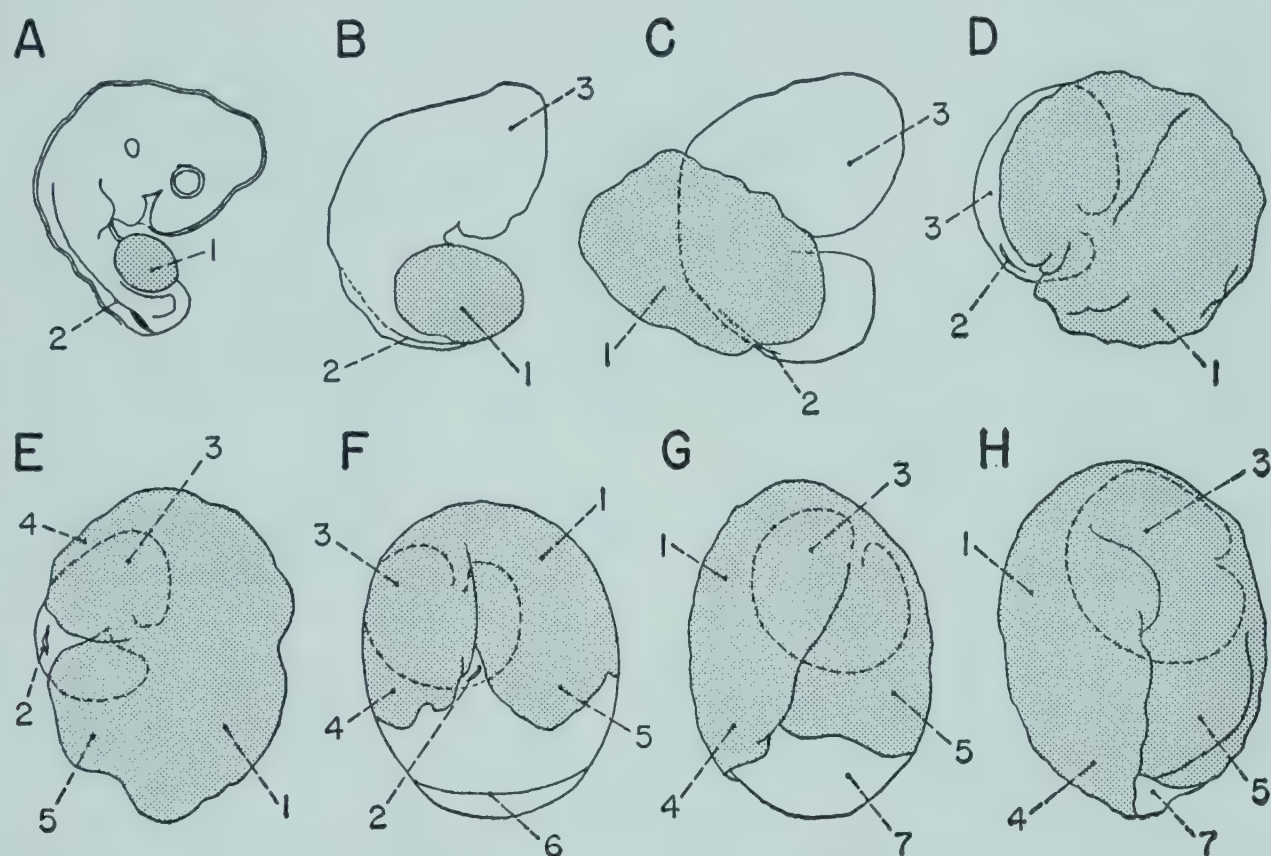


Fig. 416. The increase in the size of the allantois of the chick embryo. (Redrawn with modifications after *Hirota, 1894*.)

A, 78 hours ( $\times 2.5$ ); B, 104 hours ( $\times 2.0$ ); C, 5 days ( $\times 1.5$ ); D, 6 days ( $\times 1.0$ ); E, 7 days ( $\times 0.5$ ); F, 8 days ( $\times 0.5$ ); G, 9 days ( $\times 0.5$ ); H, 10 days ( $\times 0.5$ ).

1, allantois; 2, sero-amniotic connection; 3, amniotic cavity; 4, anterior allantoic lobe; 5, posterior allantoic lobe; 6, sinus terminalis; 7, albumen.

(*Duval, 1889*) or eighth (*Hirota, 1894*) day of development, the allantois has extended itself throughout the blunt half of the egg and has reached the equator of the yolk sac (cf. Fig. 416). It completes its enclosure of the egg's contents by the eleventh or twelfth day (*Hirota, 1894; Fülleborn, 1895*). The total surface area of the allantoic sac at the end of the tenth day is about 87 sq. cm. (*Tyrrell, Tamm, Forssman, and Horsfall, 1954*).

When the growth of the allantois is expressed in terms of the changes in its wet weight, it is seen that there is a rapid increase to a maximum on the tenth day (*Byerly, 1932; Needham, 1932a*). Wet weight then decreases somewhat until the fifteenth day but subsequently rises again, so that it



returns to the maximal value by the eighteenth or twentieth day (Fig. 417). Dry weight, both absolute and relative, rises to a maximum on the thirteenth or fourteenth day and then drops once more to a low level (Byerly, 1932).

*The Differentiation of the Chorioallantois and the Inner Allantoic Limb*

Early in the chick's fifth incubation day, the mesenchyme covering the fundus of the allantoic sac comes into contact with the mesenchyme lining the chorion. The point of first contact is small and is almost in the middle

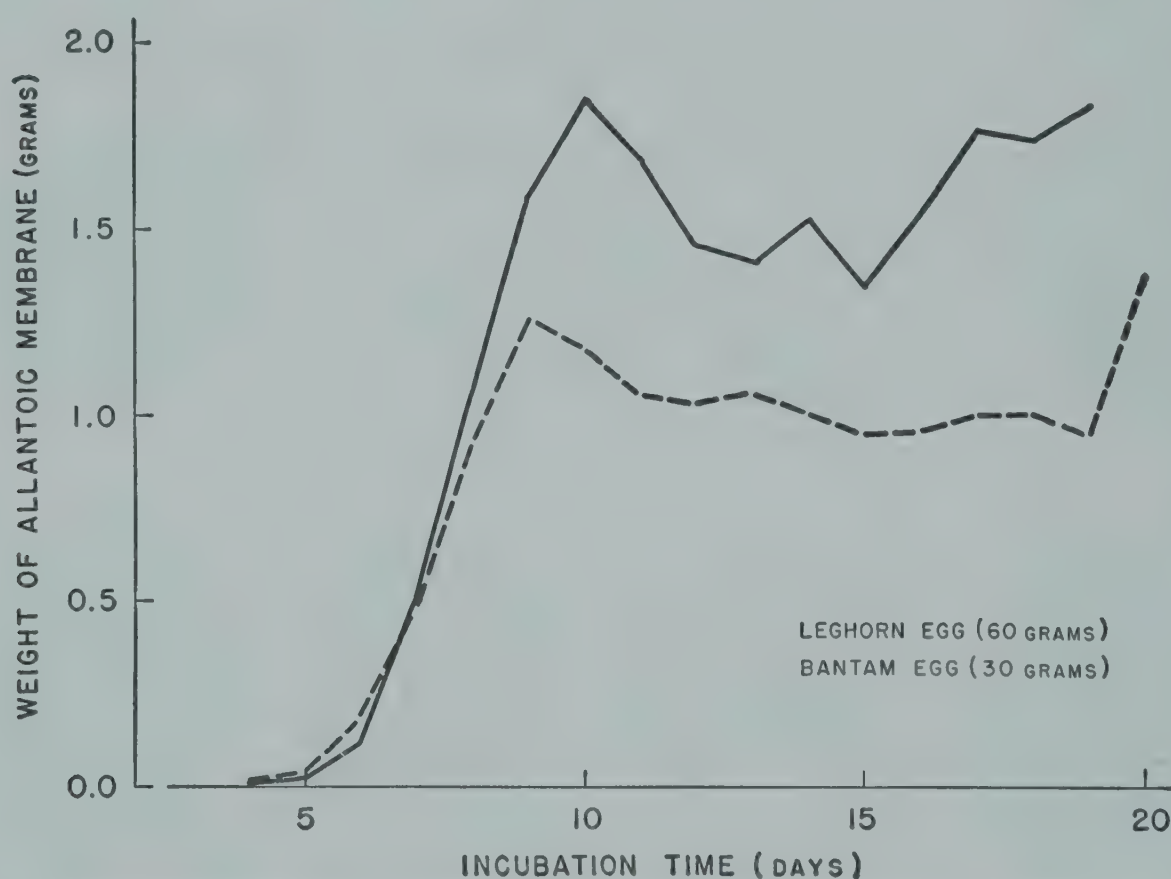


Fig. 417. Successive stages of growth of allantoic membrane as shown by its increasing weight in eggs of Leghorn and Bantam chickens, *Gallus gallus*. (Plotted from the data of Byerly, 1932.)

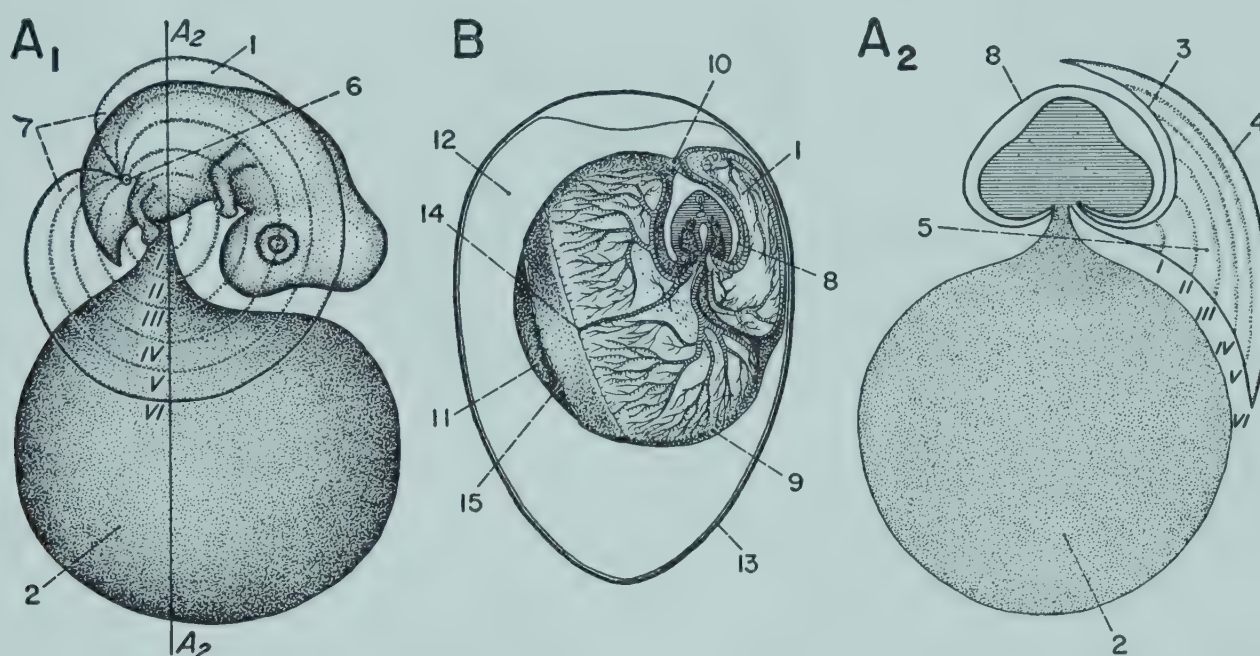
of the long axis of the egg (Steinmetz, 1930). The two membranes immediately begin to fuse and thus to obliterate the extraembryonic coelom between them. The growing allantoic sac is soon forced to flatten out beneath the chorion, which lies close to the eggshell, and the area of contact and fusion is thereby increased. As soon as the allantois begins to flatten, it is possible to distinguish an inner and an outer allantoic wall or limb, separated by the cavity of the allantois (Fig. 418-A<sub>2</sub> and B).

The outer limb, compounded of the coherent chorion and allantois, is usually known as the chorioallantois. It is the larger of the two allantoic limbs, its total area at the end of the chick's tenth incubation day being about 58 sq. cm. Failure to turn the egg between the fourth and the tenth days causes its area to be reduced by 31 per cent (Tyrrell, Tamm, Forssman, and Horsfall, 1954). After the midpoint of incubation, an extension of the



chorioallantois becomes folded around the albumen and thus participates in the formation of the albumen sac. The respiratory quotient of the chorioallantois drops from 0.95 on the fifth day to about 0.82 on the fifteenth day and thereafter (*Needham, 1933*).

The inner allantoic limb comprises about one third of the allantoic sac; its total area at the end of the chick's tenth incubation day is 29 sq. cm. (*Tyrrell, Tamm, Forssman, and Horsfall, 1954*). It contains large blood vessels, and its chief function seems to be to distribute them to the chorioallantois. Beginning on the seventh day, the inner limb fuses with



**Fig. 418.** Early stages in the development of the allantois. (*A<sub>1</sub>* and *A<sub>2</sub>*, modified after Steinmetz, 1930; *B*, redrawn with modifications after Witschi, 1949.)

*A<sub>1</sub>*, diagram to show how the allantoic vein impedes the growth of the allantoic sac, and causes its division into lobes; the circles represent the allantois at successive stages of growth. *A<sub>2</sub>*, a diagrammatic section, as indicated in *A<sub>1</sub>*; *B*, contents of sparrow (*Passer domesticus*) egg incubated 5.5 days, in which the body of the embryo has been cut off above the yolk stalk, and the allantoic development has reached a stage close to number VI shown in *A<sub>1</sub>* and *A<sub>2</sub>*.

1, allantois; 2, yolk; 3, inner allantoic wall; 4, outer allantoic wall; 5, cavity of allantois; 6, allantoic vein; 7, lobes of allantois; 8, amnion; 9, chorion; 10, chorioamniotic connection; 11, yolk plug; 12, albumen; 13, shell; 14, terminal venous sinus and upper left vitelline vein; 15, edges of ectochorion and endoderm.

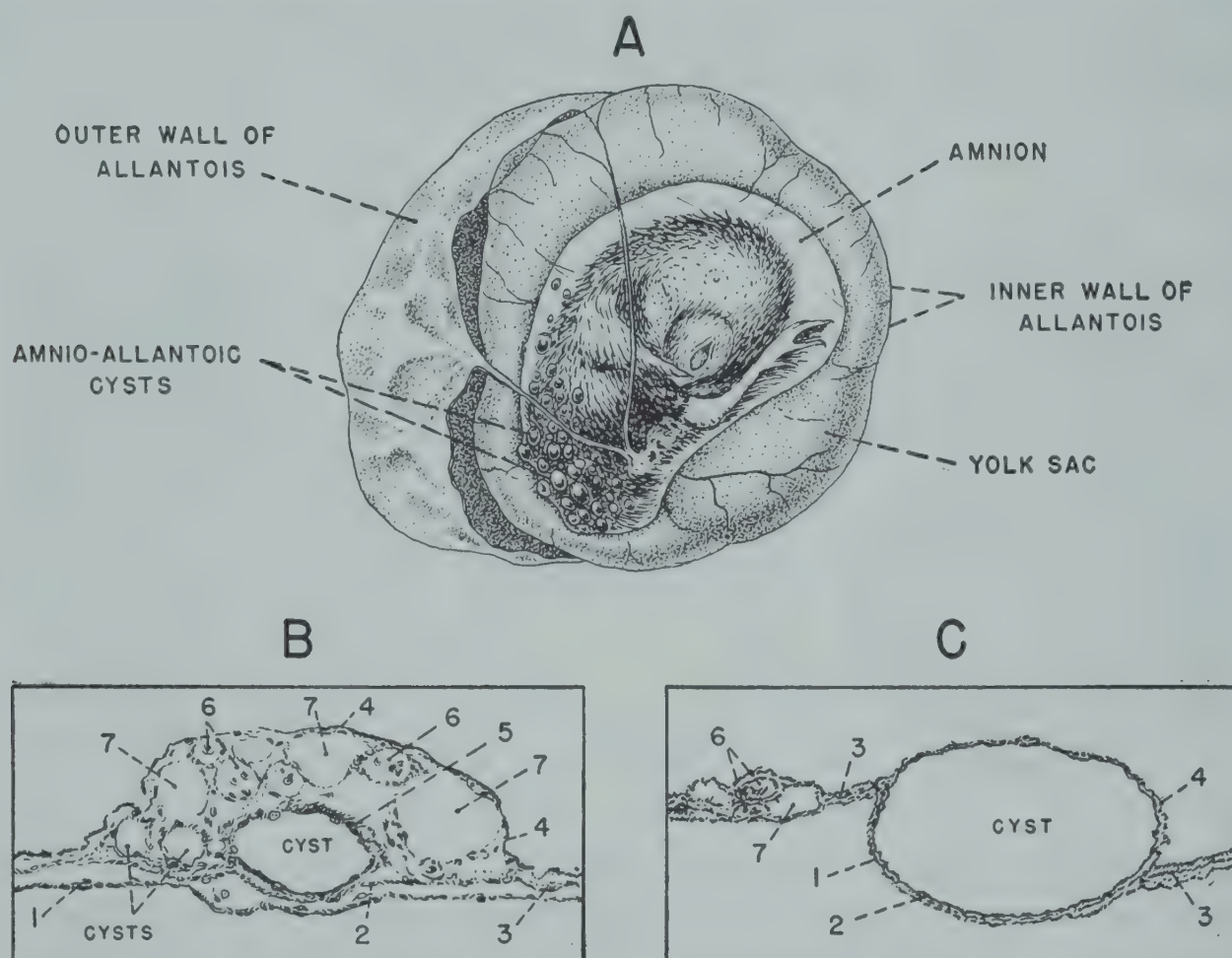
the amnion (*Virchow, 1891*) over an area that increases continually throughout incubation. Cohesion is usually so intimate that separation is impossible (*Steinmetz, 1930*). Sometimes, however, cystlike cavities appear between the amnion and allantois (*Fig. 419*) on the chick's seventh day and continue to increase in number and size until the third week (*Boyden, 1929*). *Creighton (1899)* reported the presence of albumen and a small amount of yolk between the allantois and amnion of the 18-day duck (*Anas platyrhynchos*) embryo. Near the end of incubation (on the seventeenth to nineteenth days in the chick), the inner allantoic limb



also fuses with the shrunken yolk sac in the areas where the two membranes are in contact (*Fülleborn, 1895*).

### *The Formation of Septa and Lobes*

As previously noted, the allantoic sac grows toward both poles of the egg; but its progress over the embryo's body (that is, toward the blunt pole of the egg) is impeded at the end of the chick's fifth day of incubation by



**Fig. 419.** The appearance and structure of cysts occasionally occurring within the line of fusion of the amnion and the inner wall of the allantois, in the chick embryo. (Redrawn with modifications after *Boyden, 1929*.)

A, amnio-allantoic cysts of a 14-day embryo, the outer wall is opened and pulled to the left side; B, cross section of the amnio-allantoic wall of a 13-day embryo, showing a cyst at an early stage in its formation ( $\times 100$ ); C, well-formed cyst, from a 14-day embryo ( $\times 40$ ).

1, ectoderm of amnion; 2, muscle of amnion; 3, line of fusion between amnion and allantois (muscle fibers); 4, endoderm of allantois; 5, muscle of allantois; 6, blood vessels; 7, lymphatics.

the umbilical (allantoic) vein (Fig. 418-A<sub>1</sub>). Because it grows less rapidly than the allantois itself, this vein produces an indentation in the sac in passing from the inner to the outer allantoic limb. As the allantois continues to grow toward the rump of the embryo, it is divided by the vein into two ever larger lobes, one anterior to the vein and the other posterior to it. The anterior lobe overlaps the posterior one to an extent that increases with age (cf. Fig. 416-E to H). The contiguous surfaces of the lobes fuse together to form what is known as the interallantoic septum (*Hirota,*



1894), which resembles a mesentery (*Dutrochet*, 1837; *Fülleborn*, 1895). Histologically, these surfaces are recognizable as belonging to the outer rather than to the inner allantoic limb, for they are covered with ectoderm; yet, within the septum, ectoderm is not identifiable (*Hirota*, 1894; *Fülleborn*, 1895; *Steinmetz*, 1930). The allantoic lobes advance rapidly on either side of the septum, growing past the embryo and down over the yolk sac behind it, so that eventually their leading edges are directed toward the sharp pole of the egg.

During the chick's ninth and tenth days, minor septa are formed in the allantois in the same manner as the interallantoic septum, that is, through restraint exerted by blood vessels emerging from the inner to the outer limb of the allantois. Two secondary folds appear in the interallantoic septum, one caused by the umbilical vein and the other by the right umbilical artery. A caudal branch of the left umbilical artery produces a septum in the distal portion of the posterior allantoic lobe, and a cranial branch of the left umbilical artery is responsible for the formation of a similar septum in the anterior lobe (*Hirota*, 1894; *Steinmetz*, 1930). There is considerable individual variation in the configuration of the minor allantoic septa (*Hirota*, 1894), but all the septa found in the chick's allantois have been identified in the allantoides of a number of songbirds (*Fülleborn*, 1895).

#### *The Formation of the Albumen Sac*

During the chick's ninth day of incubation, the region of the allantois that is already held back by the allantoic vein is confronted with a second barrier in the form of the sero-amniotic connection. This is located above the embryo's body at the level of the rump and is directly in line with the inner end of the interallantoic septum. The outer portion of the septum does not contract the sero-amniotic connection but is carried beyond it by the growth of the allantoic lobes, which, pushing the chorion ahead of them, soon project like the eaves of a roof (*Hirota*, 1894). At the inner end of the septum, the lobes separate when they reach the sero-amniotic connection and pass around the latter on either side (*Steinmetz*, 1930). As a result, there is created an albumen-filled "cave" (*Hirota*, 1894) whose walls and roof are formed by the medial surfaces of the allantoic lobes (*Steinmetz*, 1930), and whose floor consists of the chorion. This albumen-filled tubular channel is the albumen duct, or neck of the albumen sac (Fig. 420). Maître-Jean described the albumen duct in 1722.

Where not obstructed by the sero-amniotic connection, the allantois, growing in the extraembryonic coelom, passes the equator of the yolk and turns in toward the distal pole of the latter. It does not extend, however, to the farthest limit of the extraembryonic coelom, which, bounded by the yolk sac on one side and the chorion on the other, lies between the yolk and the mass of albumen at the small end of the egg (*Duval*, 1884*b*, 1884*c*;



Virchow, 1891; Spampani, 1905; Steinmetz, 1930). The outer limb of the allantois now starts to grow at a more rapid rate than the inner limb (Steinmetz, 1930) and, always covered by the chorion, pushes forward directly beneath the eggshell. A reduplication of the chorioallantois is thus folded around the albumen. By the eleventh or twelfth day, the chorioallantois has practically surrounded the albumen. The inner limb of this double-walled extension of the chorioallantois constitutes the large, distal, rounded portion of the flask-shaped albumen sac (cf. Fig. 420). The

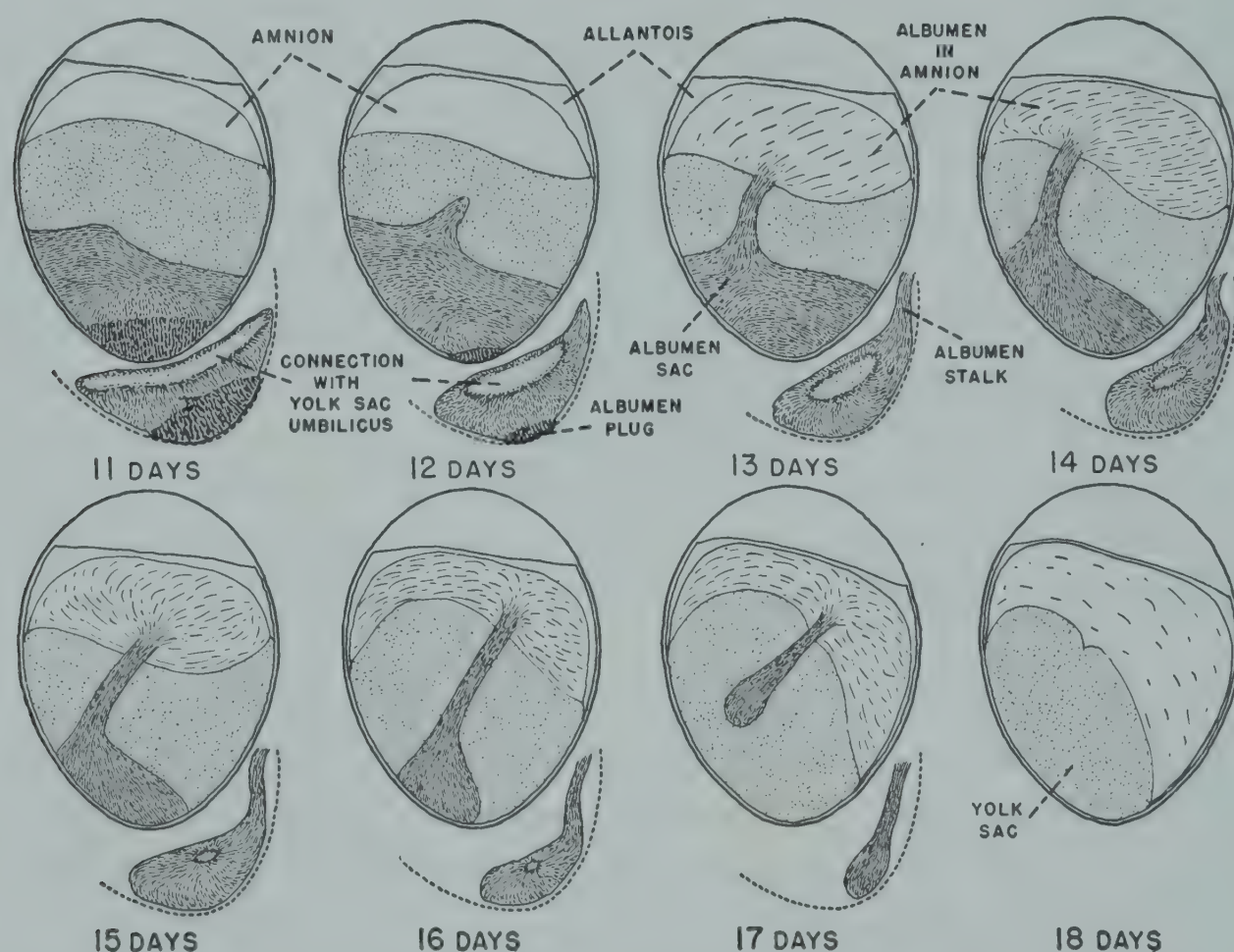


Fig. 420. General relationship of the albumen sac to the yolk sac with allantois removed and mode of transport of contents of albumen sac into amniotic cavity of the chick embryo. (Drawn after Romanoff, 1952, with elaborations.)

albumen sac is soon closed at its distal pole by the centripetal growth of the chorioallantois, as a pouch is closed when its strings are pulled (Duval, 1884b). Closure occurs on the twelfth or thirteenth day in the chick (Hirota, 1894; Fülleborn, 1895) and by the fourteenth day in the pratincole, *Glareola pratincola* (Spampani, 1905). The formation of the albumen sac seems to be delayed when the (chick) egg is left unturned during incubation (Randles and Romanoff, 1950).

Before the albumen sac closes, the sero-amniotic connection is perforated, and albumen begins to enter the amniotic cavity. As the volume of albumen decreases, the albumen sac becomes smaller, and the interallantoic septum becomes deeper at the expense of the inner limbs of the two allantoic lobes. By the sixteenth day, the albumen sac is elongated in shape (cf. Fig. 420); by the seventeenth or eighteenth day, it is a slender tube



(Hirota, 1894). Eventually, almost all the albumen is absorbed from it. A remnant of the albumen sac remains attached to the yolk sac at the yolk sac umbilicus and is taken into the chick's body at the time of hatching.

### *The Fate of the Allantois*

At hatching, most of the allantois remains in the eggshell. The small segment of the inner allantoic limb which is fused with the yolk sac is carried into the body cavity, as is the remnant of the albumen sac also (Virchow, 1891; Fülleborn, 1895). Giacomini (1893) described a small, round "allantoic body" which he found in newly hatched pigeons (*Columba livia*) situated immediately inside the umbilicus and connected with the yolk sac by means of the albumen sac. The allantoic body apparently consists of part of the inner allantoic limb together with a portion of the somatic stalk, which is inverted in following the yolk sac into the body. The allantoic body is connected with the urodaeum (the midportion of the cloaca) by means of the allantoic stalk, or urachus, which, as shown by investigations on the goose (*Anser anser*), has a swelling near its junction with the urodaeum (Rossi, 1933).

The disappearance of the residual allantoic structures after hatching is very rapid (Virchow, 1891). The urachus is transformed into a solid strand of tissue before it is resorbed (Giacomini, 1893; Rossi, 1933).

### **Histological Development of the Allantois**

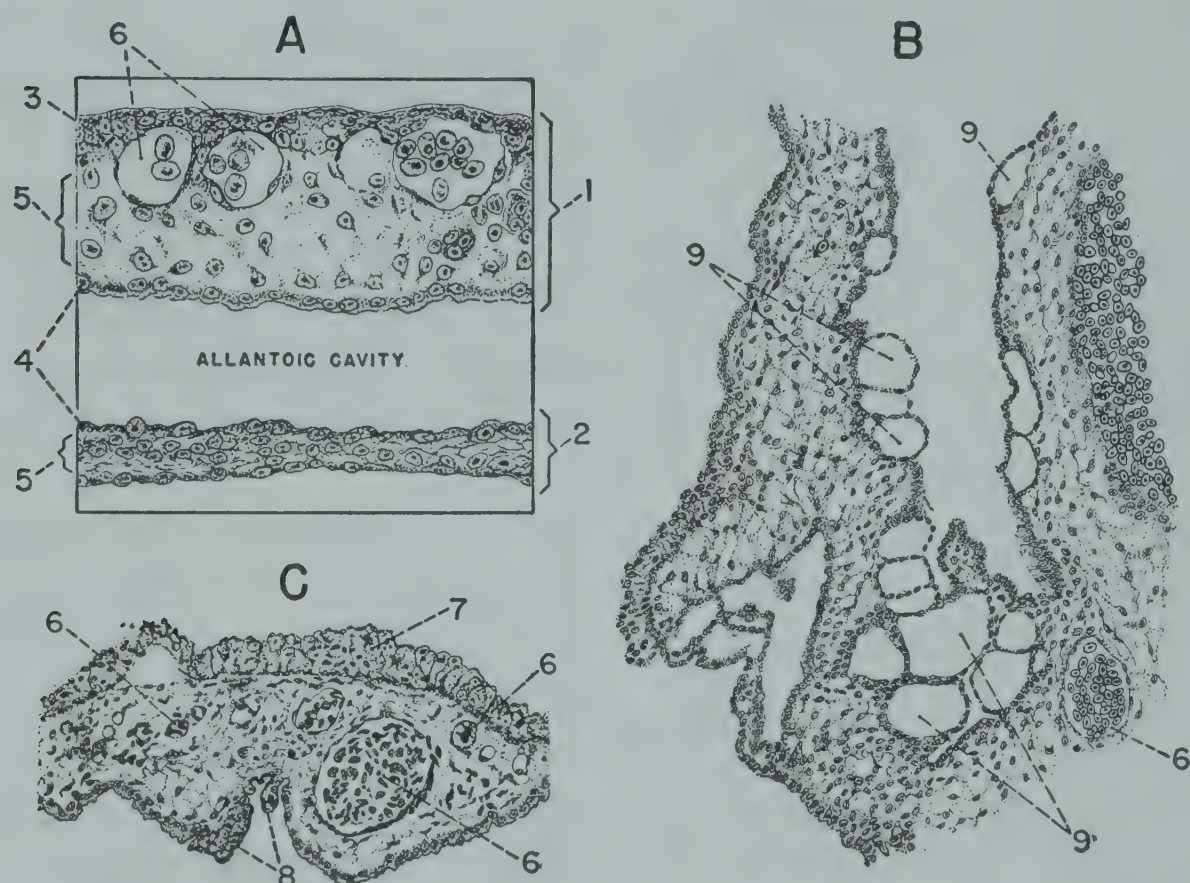
The histological structure of the allantois varies topographically, in accordance with the different functions of the three portions of the membrane (that is, the inner allantoic limb, the chorioallantois, and the albumen sac). Before coming into contact with the chorion, the allantoic sac consists throughout of an outer mesodermal component and an inner endodermal component. The mesodermal portion is composed of several layers of cells mingled with very little interstitial substance. The almost cuboidal endoderm cells are arranged in a single layer (Fülleborn, 1895). As soon as fusion begins between the allantois and the chorion, the inner and outer allantoic limbs become different histologically (Fig. 421-A).

### *The Inner Allantoic Limb*

On the chick's fifth or sixth incubation day, the inner allantoic limb has thickened in numerous places, where several layers of stellate mesodermal cells are now embedded in a homogeneous mass of interstitial substance. The outermost layer of mesodermal cells, however, is formed of flat, polygonal elements. Two days later, the initial differentiation of smooth muscle within the mesodermal component is indicated by the appearance of spindle cells which soon elongate. The muscle cells are disposed in one layer in some places and in several layers in others. Fusion of the allantoic



and amniotic musculature begins on the seventh day near the sero-amniotic connection and proceeds discontinuously at first (*Fülleborn, 1895*). At the edge of the area of amnioallantoic fusion, the allantoic muscle fibers are radially arranged (*Vulpian, 1857a*); elsewhere, they are grouped in star-shaped configurations, much as are the muscle fibers of the amnion (*Fülleborn, 1895*). On the tenth day, the individual fibers are fusiform in shape and measure 0.05 mm. in length and 0.005 to 0.008 mm. in width at the level of the nucleus (*Vulpian, 1857a, 1857b*), which is 0.014 to 0.018 mm.



**Fig. 421.** Histological structure of the allantoic sac in the avian embryo. (Redrawn with modifications A, after Boyden, 1924; B, after Rossi, 1933; C, after D'Aunoy and Evans, 1937.)

A, cross section through the inner and outer limbs of the 6-day chick (*Gallus gallus*) allantois ( $\times 200$ ); B, cross section through one of the lateral angles of the 8- to 9-day duck (*Anas platyrhynchos*) embryo ( $\times 150$ ); C, cross section of the 14-day chick chorioallantois ( $\times 100$ ).

1, chorioallantois; 2, inner allantoic limb; 3, ectoderm; 4, endoderm; 5, mesoderm; 6, blood vessels; 7, proliferative ectoderm; 8, proliferative endoderm; 9, vesicles in endoderm.

long and 0.004 mm. wide (*Vulpian, 1857b*). During the second half of incubation, the musculature of the fused amnion-allantois undergoes involution, leaving only the amniotic ectoderm and the allantoic endoderm. Involution probably occurs by resorption (*Fülleborn, 1895*). Muscle fibers have also been seen on the eighteenth day in the portion of the inner allantoic limb which fuses with the yolk sac during late developmental stages (*Vulpian, 1857a*). The allantoic muscles, like the amniotic, are non-innervated, for no nerves are present in the allantois (*Vulpian, 1857a*).



The allantoic musculature can contract independently of the amnion. Vulpian (1857*a*, 1857*b*) found that contractility in response to stimuli (such as contact, heat or cold, or galvanic currents) develops between the chick's eighth and eleventh days, is most marked from the twelfth to the fourteenth day, and persists as late as the eighteenth day. Contraction may be limited to the point stimulated, or, if a stronger stimulus is employed, may spread to the neighboring regions and may even involve the amnion.

The endoderm lining the allantoic cavity may proliferate to two or three layers of cells in some places while remaining only one layer thick in others (Rossi, 1933). Groups of large cells may alternate with groups of small ones (Fülleborn, 1895). With the aid of the phase contrast microscope, it has been determined that there are about  $3.0 \times 10^5$  cells in every square centimeter of free endodermal surface in the inner limb of the 10-day chick embryo's allantois (Tyrrell, Tamm, Forssman, and Horsfall, 1954). The total number of endodermal cells on the entire internal surface of the 10- to 12-day allantoic sac has been variously estimated, by both direct and indirect methods, as between ten million and one hundred million (Henle and Henle, 1949; Henle, 1950, 1953; Cairns and Edney, 1952; Fazekas de St. Groth and Cairns, 1952; Fazekas de St. Groth and Edney, 1952; Fazekas de St. Groth, Isaacs, and Edney, 1952; Tyrrell, Tamm, Forssman, and Horsfall, 1954). Some of the endodermal cells secrete mucus (Rossi, 1933).

In the embryo of the goose (*Anser anser*), numerous round vesicles have been seen to form in the midst of the thicker regions of the allantoic endoderm. The vesicles originate as fissures between the cell layers and are first visible on the sixth or seventh day of the 28-day incubation period. As they grow, they protrude outward into the allantoic cavity and inward toward the underlying mesenchyme (cf. Fig. 421-B). The phenomenon of vesicle formation reaches its maximum intensity on the ninth or tenth day; thereafter, it diminishes rapidly but does not cease entirely. It has been suggested that the appearance and eventual rupture of these vesicles are related to the enlargement of the allantoic cavity (Rossi, 1933).

Involution of the allantoic endoderm in the later stages of development seems to be associated with another process of vesiculation. Its initial stage, seen in the goose's (*Anser anser*) allantois after the nineteenth day of incubation (Rossi, 1933), consists of intracellular vacuolization, which has also been observed in the chick's allantois (Fülleborn, 1895). Certain superficial cells of the endoderm, either singly or in groups, become hydropic and spheroidal, with clear cytoplasm and deformed and displaced nuclei. As the numbers of such cells increase, they protrude into the allantoic cavity, and irregular spaces appear between them and the deeper elements. As aggregates of the superficial cells degenerate and fall into the allantoic cavity, depressions are left in the epithelium, but these are soon filled with new cells. The degenerative process increases in intensity, with



the result that the endodermal epithelium which lines the goose's (*Anser anser*) allantois immediately before hatching contains many vacuolated and hydropic cells and appears to be studded with small buds. Most of the cells, however, have retained their cuboid form, and the epithelium is still pluristratified (Rossi, 1933).

Inoculation directly into the allantoic cavity became a widely used technique in the culture of a number of viruses (Cox, 1952) after it was discovered (Burnet, 1941) that the influenza virus multiplies in the endodermal cells lining the allantoic sac.

### *The Chorioallantois*

When the outer mesodermal layer of the allantois fuses with the mesodermal lining of the chorion to form the chorioallantois, the chorionic ectoderm and the allantoic endoderm are also incorporated into the new membrane, which is thus composed of three germ layers. With continued incubation, some modification of histological structure normally occurs in the chorioallantois. Various experimental procedures may greatly alter the microscopic appearance of the membrane.

As the area of chorioallantoic fusion increases, changes can be seen in the ectoderm everywhere except in a zone of varying width at the outer edge of the fused region. The cells rapidly lose their original cuboidal character, become extremely flat, and dispose themselves in a single layer (Fülleborn, 1895). By the tenth day, one or two additional layers of cells have been added beneath the superficial flat elements. Capillaries, formerly confined to the mesoderm, are now present in the midst of the ectoderm (Danchakoff, 1917). By the thirteenth to fifteenth day, the outermost flat cells have disappeared (Fülleborn, 1895), and the persisting ectodermal elements, which are cuboidal, lie below the terminal capillaries of the respiratory network (Danchakoff, 1917).

Variations in the appearance of the presumably normal chorioallantoic ectoderm have been described. After the tenth or eleventh day, the ectoderm may proliferate in patchy areas of varying size, until it is eight or more cells thick. The cells are greatly enlarged and arranged irregularly; many contain vacuoles and small and large inclusions. Sometimes proliferative areas from the ectoderm project into the mesoderm. The phenomenon of ectodermal proliferation, which may or may not be accompanied by mesodermal and endodermal proliferation, reaches a maximum by the chick's thirteenth or fourteenth incubation day (Fig. 421-C) and is less frequently seen after the fifteenth day. At this time, however, the ectoderm may begin to exhibit papillary projections (D'Aunoy and Evans, 1937).

The mesoderm of the chorioallantois is an embryonic connective tissue transversed by blood vessels. Its thickness varies considerably (Goulston and Mottram, 1932) and is usually greatest around the large vessels



(D'Aunoy and Evans, 1937). Until the middle of the second week of incubation, its outermost portion contains the respiratory network of capillaries. As early as the end of the sixth day, this network is so dense that the mesoderm directly below the ectoderm is not so much a tissue traversed by a vascular plexus as it is a blood sinus interrupted by mesodermal trabeculae (Fülleborn, 1895). It is said that the mesoderm of the normal chorioallantois is often thickened in small to large areas because of cellular proliferation, which is sometimes associated with edema. Ectodermal proliferation and endodermal hypertrophy may occur in the same areas. The edematous and proliferative conditions of the mesoderm are maximal at the thirteenth to fifteenth days of incubation and then disappear rapidly (D'Aunoy and Evans, 1937).

The endoderm of the chorioallantois is not essentially different from that of the inner allantoic limb, which has already been described. The cells, however, are larger, for the average number per square centimeter of endodermal surface is  $1.66 \times 10^5$  in the 10-day chick's chorioallantois, as compared to  $3.0 \times 10^5$  in the inner limb (Tyrrell, Tamm, Forssman, and Horsfall, 1954). Moderate proliferation of the endoderm has been seen in limited regions, accompanying mesodermal and ectodermal proliferation. The endodermal cells in these regions may contain inclusions (D'Aunoy and Evans, 1937).

**Injury Reactions in the Chorioallantois.** The chorioallantois has been found to exhibit a characteristic local reaction wherever the eggshell is removed and only the shell membranes are left as protection. Within a short time (but at least 2 hours) after removal of the shell, the chorioallantois beneath the exposed shell membranes may hypertrophy, or it may become atrophic and appear transparent and thinner than normal (Goulston and Mottram, 1932; Hunt, 1934; Goldsworthy and Moppett, 1935). The hypertrophic reaction may involve only ectoderm, or this layer plus either mesoderm alone or mesoderm and endoderm. The subshell capillaries are always absent. If the mesoderm is hypertrophied, the ectoderm is usually necrotic and shows evidence of previous hypertrophy. Endodermal hypertrophy is accompanied by necrosis of both mesoderm and ectoderm (Goulston and Mottram, 1932). Sometimes thickening of the chorioallantois is limited to the periphery of the "window" in the shell (Moppett, 1933; Hunt, 1934; Goldsworthy and Moppett, 1935). The peripheral reaction consists of a papillomatous hypertrophy of the endoderm, with less severe changes in the other two germ layers. The endodermal cells are swollen and irregular and contain large vacuoles and inclusions (Goldsworthy and Moppett, 1935). When the atrophic reaction occurs, it is usually found in the central part of the exposed area. It is characterized by absence of blood vessels and death and disorganization of the tissue, which consists only of a thin layer of necrotic cells (Goulston and Mottram,



1932). Sometimes a small region where all germ layers are hypertrophic is found at the edge of such an atrophic area (*Goulston and Mottram, 1932; Moppett, 1933; Goldsworthy and Moppett, 1935*). The hypertrophic and atrophic reactions are probably expressions of different degrees of injury affecting successively deeper levels of the chorioallantois, atrophy being the end result of injury at all levels (*Goulston and Mottram, 1932*).

Since absence of the calcareous shell for a short time seems to be all that is necessary to produce the reactions described above, it appears likely that they are caused by a local increase in evaporation; and, in fact, it has been observed that pronounced atrophic reactions may occur after a blast of warmed, filtered air has been directed upon a shell window for 10 minutes. Hypertrophy at the periphery of a window is perhaps the result of increased permeability of the shell membranes in this location, where the greatest strain is placed upon them at the time the shell is removed (*Goldsworthy and Moppett, 1935*). An injury by incision or cautery usually causes only a temporary proliferation of the chorioallantoic mesenchyme (*Danchakoff, 1918*).

Hypertrophy or atrophy is supposedly caused by X-rays acting on the chorioallantois through a shell window, but it is difficult to separate the effects of radiation from the effects of mere removal of the shell. Moppett (1933), however, reported that homogeneous X-rays caused hypertrophy and (in larger doses) atrophy in an area whose shape reproduced the outline of the incident beam, and he also found that direct mixed rays applied through the intact eggshell caused hypertrophy of the chorioallantoic mesoderm and endoderm, followed by autolysis and atrophy. A 3- to 4-hour exposure to gamma rays from 6.0 mg. of radium has no effect (*Goulston, 1932*), but an exposure of 24 hours or more has been seen to produce an atrophic reaction not observed in membranes beneath shell windows in control eggs (*Hunt, 1934*). Exposure for half an hour or more to beta rays from 60.0 mg. of radium shows that the vascular system of the chorioallantois is especially sensitive to radiation. The capillary network is destroyed and the deeper blood vessels are thrombosed over an area that varies in size according to the exposure time. Within this area, there is a central dead region where all three germ layers have degenerated, probably because of direct action on the cells. This region is surrounded by a narrow zone where degeneration is accompanied by hypertrophy, which is probably a secondary injury reaction (*Goulston, 1932*).

**Reactions to Infections.** The extensive use of the chick's chorioallantois for the culture of viruses and bacteria was foreshadowed by the experiments of Rous and Murphy (1911) but may be said to have begun when the advantages of the method were pointed out by Woodruff and Goodpasture (1931), Goodpasture (1933), and Goodpasture and Anderson (1937).



The histological reactions of the membrane to virus infections vary according to the type of virus. Some viruses multiply and produce visible foci on the chorioallantois, whereas others produce only weak foci or require adaptation to create definite lesions; still others may grow without causing any lesions at all (Cox, 1952). Particular viruses have a greater affinity for certain types of cells than for others; therefore the various germ layers of the chorioallantois are affected to different degrees by different viral agents. Among the characteristic changes which may occur in the infected cells are hyperplasia and hypertrophy and the development of inclusion bodies, as well as such processes as swelling, vacuolation, degeneration, lysis, and necrosis (Buddingh, 1952). As D'Aunoy and Evans (1937) noted, some of these phenomena may be found in presumably normal chorioallantoic membranes, particularly between the tenth and fifteenth days of incubation; therefore interpretation of the effects of viruses must be made with care.

Bacteria inoculated on the chorioallantois produce lesions that are not only characteristic for the bacterial type but also simulate the lesions found in the natural host. Microorganisms may multiply on the surface of the membrane or may invade it and grow intracellularly. Like viruses, bacteria exhibit differing affinities for the germ layers of the chorioallantois (Goodpasture and Anderson, 1937). Pathogenic fungi may also produce lesions on the chorioallantois that are comparable to those of the natural disease (Moore, 1941).

**Reactions to Tissue Grafts.** The ability of the chick's chorioallantois to support the growth of living tissues placed in contact with it was shown by the pioneering work of Murphy (1913), Danchakoff (1916, 1918), Kiyono and Sueyasu (1917), Minoura (1921), and Atterbury (1923). The technique of chorioallantoic grafting has since proved invaluable in the study of such problems as embryonic differentiation, the localization of early developmental potencies, the effect of hormones on development.

The presence of a tissue fragment on the chorioallantois causes a localized hypertrophy of the membrane, which may reach 1.0 mm. in thickness (Huxley and Murray, 1924). The first reaction is usually manifested by the ectoderm in contact with the graft. If a graft is placed in position on the seventh to ninth days of incubation before the capillary network of the membrane has invaded the ectoderm, the cells of this germ layer begin to proliferate rapidly. Mitoses are numerous, and many multinuclear cells are formed. Immediately surrounding the graft, the ectodermal cells become aggregated into papillae and change from cuboidal to fusiform as the papillae penetrate into the implant. The proliferative process continues in later stages, with the result that the originally double layer of cuboidal cells evolves into a stratified epithelium extending for a considerable distance around the graft. If grafting is not performed until the eleventh or twelfth



incubation day, when the capillary network has reached a superficial position, pressure exerted by the piece of tissue ruptures the walls of the capillaries, so that hemorrhages occur. The ectoderm is broken up into groups of cells, and the graft passes through into the mesodermal layer, carrying ectodermal fragments with it. The cells of the ectodermal islands continue to proliferate and form solid strands and cornified pearls (*Danchakoff, 1918*). The pearls consist of a stratified epithelium around a core of concentric layers of keratin. The stratified ectoderm at the surface may also undergo cornification (*Huxley and Murray, 1924*).

Grafts to the chorioallantois are able to survive because of the highly vascular nature of the membrane (*Willier, 1924*). Capillaries grow into implants very rapidly, and vascular connections are often established in 24 hours (*Minoura, 1921*). The optimum time for engrafting tissue is from the ninth to twelfth day of incubation (*Danchakoff, 1918; Minoura, 1921; Willier, 1924*), for it is during this period that the capillary network of the chorioallantois is approaching the surface; grafts made earlier may not survive because the ectoderm sometimes protects the layers beneath it so well that vascularization and incorporation of the graft does not occur (*Danchakoff, 1918*). A successful graft is usually found in the mesodermal layer of the membrane, with blood vessels converging toward it (*Minoura, 1921; Willier, 1924*). Around the blood vessels in the immediate vicinity of the graft the mesenchymatous stroma is greatly increased in quantity, though somewhat looser in structure than normal (*Minoura, 1921*); this increase in the mesodermal component of the chorioallantois largely accounts for the formation of a mass around a graft (*Huxley and Murray, 1924*). A remarkable feature characterizing the response of the chorioallantoic mesenchyme to the presence of certain tissues from adult chickens is the pronounced proliferation of immature leucocytic elements, a process which—like the appearance of blood islands in cultures of the allantois *in vitro* (*Katzenstein, 1925*)—reveals latent hematopoietic potencies (*Danchakoff, 1918*). This response is part of a generalized one which occurs throughout the embryo after the grafting of such adult homologous organs as spleen (*Murphy, 1916; Danchakoff, 1918*), liver (*Murphy, 1916; Willier, 1924*), kidney, bone marrow (*Murphy, 1916*), thyroid, and thymus (*Willier, 1924*). Until the eighteenth day of incubation, the chick's chorioallantois supports the growth of tissues from foreign species, but after this time the connective tissue elements of the chorioallantoic mesenchyme increase markedly about a heteroplastic graft and gradually invade and replace the implanted tissue (*Murphy, 1914*).

The endoderm of the chorioallantois is probably not directly affected by a graft and, in fact, may not exhibit any reaction at all. A response, when it occurs, may be delayed until 2 or 3 days after the implantation of tissue. The endodermal cells become less flat and undergo proliferation to several



layers of almost cylindrical epithelium (*Danchakoff, 1918; Huxley and Murray, 1924*).

**Permeability of Chorioallantoic Tissue.** The permeability of the various layers of the chorioallantois has an important bearing on the function of this membrane (as an integral part of the allantoic sac) in the storage of excretory products. Membrane permeability is also a factor determining the distribution of substances introduced into the egg in the course of experimental investigations.

The colloidal dye, trypan blue, is not absorbed equally well by all types of chorioallantois cells. When introduced into the air chamber of the chicken egg (*Atwell and Hanan, 1926; Hanan, 1927; Latta and Busby, 1929*), the dye soon passes through the inner shell membrane and into the chorioallantoic ectoderm, either by way of the albumen (between the fourth and seventh days of incubation) or directly (after the seventh day). The ectodermal cells become laden with blue granules of various sizes. The mesenchymal cells may stain diffusely or may store the dye in the form of fine grains. Later, some of the mesenchymal cells become polymorphonuclear and mononuclear phagocytes and enter the blood and lymphatic vessels. Probably dye from the mesenchymal interspaces also finds its way into the blood stream by passing directly through the vascular endothelium; the latter, unlike the lymphatic endothelium, does not retain the dye. Dye excreted by the kidneys of the vitally stained embryo colors the allantoic fluid blue. In general, the endodermal cells remain unstained, as they usually do when trypan blue is injected directly into the allantoic cavity (*Wislocki, 1921; Taylor and Saenz, 1949*). Sometimes, however, injection of this substance into the allantoic sac may result in vacuolation and cytoplasmic staining of a varying proportion of the endodermal cells. Mesenchymal elements then may take up the dye and carry it to the embryo, although not in sufficient quantities to cause gross vital staining (*Latta and Busby, 1929*).

Soluble radioactive phosphorus ( $P^{32}$ ), injected as dibasic sodium phosphate into the allantoic cavity of the 11-day chick embryo, tends to be retained there. When relatively large amounts of the isotope are injected, traces of it are sometimes detectable in the blood and embryonic tissues 2 or 3 days later. In younger (8-day) embryos, slightly better absorption may occur (*Taylor and Saenz, 1949*). Retention of phosphates in the chick's allantoic cavity is also indicated by various physical properties (such as osmotic pressure,  $pH$ , and electrical conductance) of the allantoic fluid after the injection of 0.5 milliliter of a molar phosphate buffer into the allantoic sac on the eighth day of incubation (*Randles, 1951*).

Promin and sulfathiazole, on the other hand, apparently pass through the endoderm of the membrane with ease, for they are detectable in the embryo's blood stream 30 minutes after being injected into the allantoic cavity.



These substances, as well as streptomycin (*Lee, Stavitsky, and Lee, 1946*), rabbit hemolysin, and guinea pig serum complement (*Polk, Buddingh, and Goodpasture, 1938*), may appear in the blood or the allantoic fluid, or both, 12 to 24 hours after being dropped on the external surface of the chorioallantois. Since absorption by this route was demonstrated in 10- to 16-day embryos, it cannot be stated whether the chorioallantoic ectoderm or only the epiectodermal capillaries participated in the process.

### *The Albumen Sac*

Essentially, the albumen sac is merely a fold of the chorioallantois, and it is thus an integral part of the allantoic sac. Certain features of its histological structure nevertheless distinguish it from the remainder of the allantois.

As Fülleborn (1895) remarked, the albumen sac is of variable thickness but is thicker, in general, than the inner allantoic limb. Its endoderm resembles that found elsewhere in the allantoic sac, but the cells are perhaps less flat. Its mesodermal component contains blood and lymph vessels, but possesses less interstitial substance than the corresponding component of the chorioallantois. Adjacent to the ectoderm, the mesoderm forms a compact stratum consisting of several layers of spindle cells. Close to the yolk sac umbilicus, the chorion and the outer allantoic limb do not come in contact with each other, and the albumen sac in this region is composed, therefore, of chorion alone. Mesodermal elements resembling smooth muscle cells have been seen in the chorionic portion of the albumen sac, radiating from the yolk sac umbilicus (*Fülleborn, 1895*).

The ectodermal component of the albumen sac forms its lining and consists of various types of cells—flat, cuboidal, and cylindrical (*Virchow, 1891; Fülleborn, 1895; Steinmetz, 1930*). In describing the albumen sac of the coot (*Fulica atra*), Steinmetz (1930) stated that the ectoderm is composed of an outer stratum of flat cells and an inner stratum of taller cells. The inner stratum forms peculiar villus-like projections which extend into the albumen (*Duval, 1884b, 1884d, 1884e*) especially in the region nearest the yolk sac umbilicus, but they contain no connective tissue or blood vessels (*Virchow, 1891; Fülleborn, 1895; Creighton, 1899; Spanpani, 1905; Steinmetz, 1930*). Similar structures have been seen in albumen sacs of songbirds (*Duval, 1884e; Fülleborn, 1895*). The ectodermal protuberances in the albumen sac of the 13-day coot (*Fulica atra*) embryo (Fig. 422-A) are fairly thick-walled but hollow (*Steinmetz, 1930*), and, as in the chick's albumen sac (*Virchow, 1891*), their cavities are filled with a substance whose staining reactions resemble those of egg albumen. By the coot's 16-day stage, these structures have become thinner-walled and have spread farther distally. Ectodermal projections of a second type have now appeared; they are higher, narrower, and more pointed than the first type and



are composed of densely packed cells (Fig. 422- $B_1$ ). At this time the entire epithelial lining of the albumen sac is covered with a thin layer of vacuoles (Fig. 422- $B_2$ ) of uncertain origin which have differentiated in the albumen (Steinmetz, 1930). The presence of these vacuoles, as well as the inclusion of egg albumen within the ectodermal protuberances, makes it appear probable that the albumen sac may play an active but not very important role in the absorption of albumen (Virchow, 1891; Steinmetz, 1930).

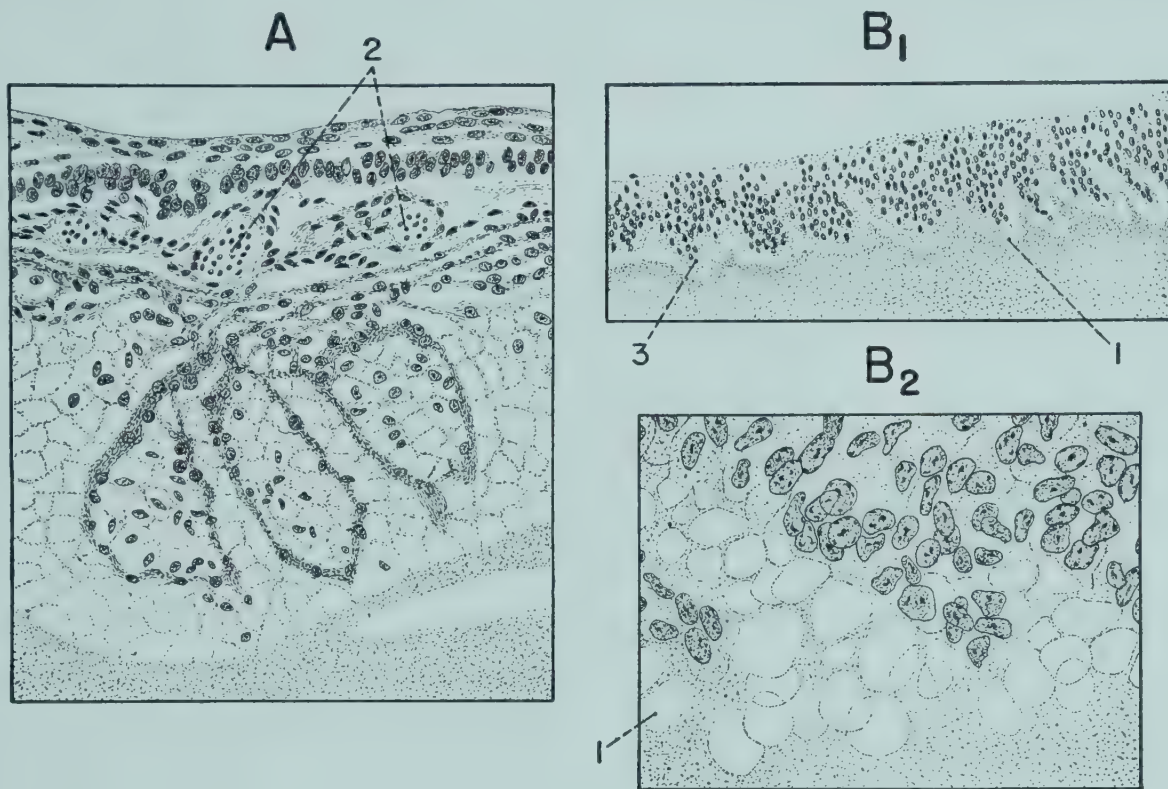


Fig. 422. Histological structure of the epithelial lining of the albumen sac in the developing embryo of the coot, *Fulica atra*. (Redrawn by the author with modifications after Steinmetz, 1930.)

A, 13-day embryo ( $\times 450$ );  $B_1$ , 16-day embryo ( $\times 170$ );  $B_2$ , 16-day embryo ( $\times 2300$ ).

1, vacuole; 2, blood vessels; 3, ectodermal projections.

### The Circulatory System of the Allantois

The allantois is extremely well vascularized. This is not surprising, since its outer limb, the chorioallantois, performs the function of a lung during embryonic life. The chorioallantois is characterized by the presence of a truly respiratory type of capillary network (Dalrymple, 1844) situated in an advantageous position directly beneath the shell membranes, as close as possible to the source of oxygen. The blood vessels from which this luxuriant capillary plexus is derived leave the embryo's body via the allantoic stalk and branch in the inner allantoic limb before passing on to the chorioallantois. There are three definite allantoic blood vessels, the (left) allantoic or umbilical vein and the right and left allantoic or umbilical arteries. The developmental history of the intraembryonic portions of these vessels is given in Chapter 8. The allantois also possesses a system of lymphatic vessels continuous with the embryonic lymphatic system.



*The Large Blood Vessels*

The allantois is vascularized almost from the beginning. As Duval (1877) noted, blood vessels (the terminal branches of the umbilical arteries) are present in the mesodermal portion of the allantoic primordium of the 60-hour chick embryo. The umbilical arteries are paired within the embryo's body, then join to form a single stem while passing through the umbilical cord to the allantois, where the stem once more divides into right and left branches (Kutsuna, 1933). During the third day, the allantois is drained not by the umbilical veins but by bilateral branches of the subintestinal vein which empty into the omphalomesenteric (vitelline) veins. On the fourth day, connections are established with the umbilical veins, and those with the subintestinal vein are quickly lost. The subintestinal and right umbilical veins then disappear (see Chapter 8).

The large definitive blood vessels are clearly seen in the allantois of the 5-day coot (*Fulica atra*) embryo (Steinmetz, 1930). The umbilical vein is unbranched and lies close to the small right artery. In the inner allantoic limb, the left artery courses for a considerable distance parallel to the long axis of the egg, and then divides into a cranial and a caudal branch running almost perpendicular to the main stem of the artery. Both of these branches, as well as the umbilical vein, are long enough to extend on to the outer limb of the allantois. A similar stage of development is seen in the 104-hour chick embryo (Hirota, 1894).

In the 6-day coot (*Fulica atra*) embryo, the umbilical vein courses for a short distance in the portion of the inner allantoic limb which is growing over the body of the embryo; then, reversing its direction, it passes to the outer limb, dividing the allantois into two lobes as it does so (Steinmetz, 1930). Fülleborn (1895), in describing the allantoic vessels of the 6-day chick embryo, stated that the umbilical vein gives off branches accompanying both arteries in the inner limb, as well as other branches in the inner limb and in the interallantoic septum. At the 6-day stage, the right umbilical artery of the coot embryo lies close to the vein but does not leave the inner limb. The left artery, on its way to the outer limb, passes through the portion of the inner limb which lies above the yolk sac (Steinmetz, 1930). In the outer limb, extensive arterial and venous branching occurs. The larger vascular stems send out lateral branches to either side, like the twigs of a fir tree, and this type of division continues until vessels of capillary caliber are given off. The venous and arterial vessels can be seen to interdigitate with each other in the 6-day chick embryo (Fülleborn, 1895), and the 8- to 9-day coot embryo (Steinmetz, 1930); Fig. 423 shows the regularity of their arrangement later in the incubation period. Through the extensive anastomosis of small arterial and venous twigs with others of the same type, there is established an arterial and a venous network the



meshes of which cross one another (*Fülleborn, 1895*). The capillaries connect the venous and arterial networks (*Giacomini, 1930a, 1930b*).

In the allantois of the 8- to 9-day coot (*Fulica atra*) embryo (Fig. 423-A<sub>1</sub> and A<sub>2</sub>) the umbilical vein divides into a caudal and a cranial branch shortly after entering the outer allantoic limb; these branches, like those already given off by the left artery in the inner limb (cf. Fig. 423-A<sub>1</sub> and A<sub>2</sub>), parallel the transverse axis of the egg. By this time, the left artery has sent out two additional lateral branches in the inner limb (*Steinmetz, 1930*).

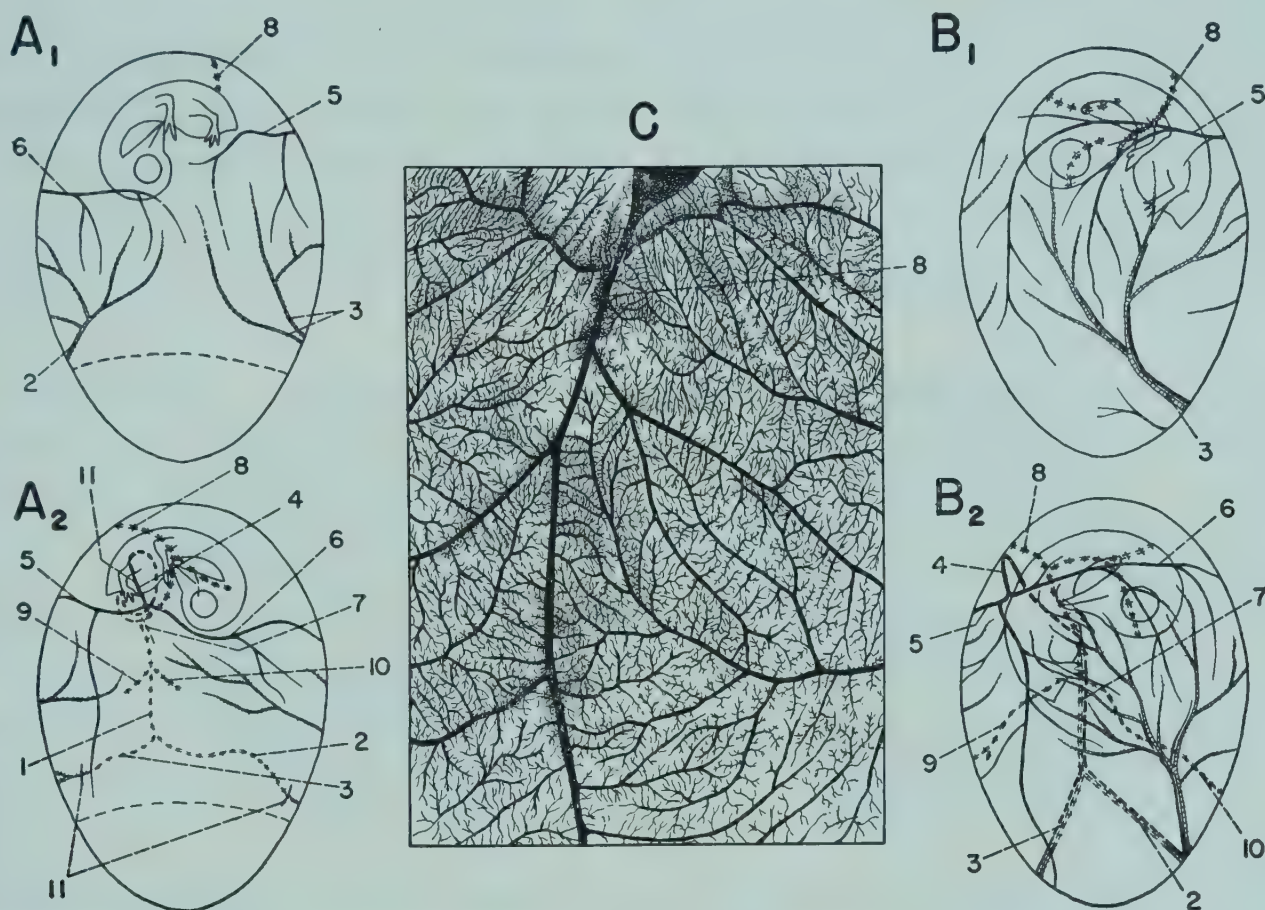


Fig. 423. Stages in the development of the large blood vessels in the allantois of the embryonic coot, *Fulica atra*. (Redrawn with modifications after *Steinmetz, 1930*.)

A<sub>1</sub>, A<sub>2</sub>, allantoic vascular system on the ninth incubation day, from the right and left sides (with reference to the embryo's body). The veins are shown black, the arteries light and crosshatched, vessels in the inner allantoic limb are represented by broken lines ( $\times 0.5$ ); B<sub>1</sub>, B<sub>2</sub>, the same, on the fifteenth day of incubation ( $\times 0.5$ ); C, interdigitating allantoic blood vessels on the seventeenth day of incubation ( $\times 2$ ).

1, left allantoic artery; 2, 3, main branches of the left allantoic artery; 4, allantoic vein; 5, 6, main branches of the allantoic vein; 7, branch of the allantoic vein accompanying the left allantoic artery; 8, right allantoic artery; 9, 10, branches of the left allantoic artery in the inner limb of the allantois; 11, site of septum in the allantois.

In older embryos (*Fülleborn, 1895*), as exemplified by the 14- to 15-day coot, *Fulica atra* (*Steinmetz, 1930*), the branch of the umbilical vein accompanying the left artery is greatly increased in diameter and in length (Fig. 423-B<sub>1</sub> and B<sub>2</sub>). It follows this artery throughout both the inner and the outer allantoic limbs, branching wherever the artery branches. The artery now ramifies much more extensively, especially in the inner limb. The right artery persists until close to the end of incubation but is of sub-



ordinate significance during the second half of development; it supplies the interallantoic septum and part of the inner allantoic limb (Fülleborn, 1895).

Conditions of the atmospheric environment affect the development of the allantoic blood vessels. Flemister and Cunningham (1940) incubated chicken eggs at an atmospheric pressure of 40 lb. and, at the end of 10 days, found that the larger allantoic vessels had progressed only to the stage normally reached after 8 days. Failure to turn incubating eggs also retards the growth of these vessels (Romanoff and Randles, 1954). In eggs left unturned for the entire developmental period, the chorioallantoic vessels remain in the same position for so long that grooves may form opposite them on the inner surface of the eggshell (Fig. 424). These grooves are evidence of the role played by the allantoic circulation in the mobilization of calcium from the eggshell (Glaser and Piehler, 1934).

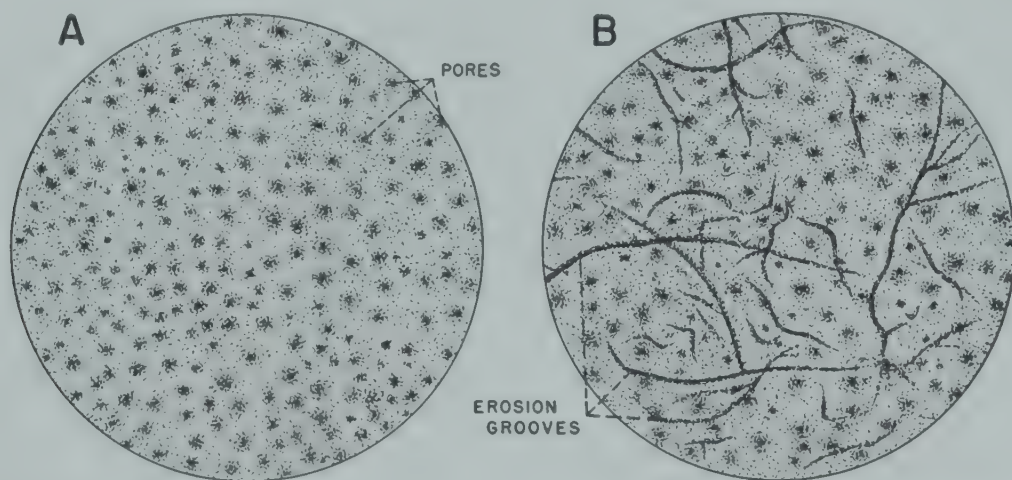


Fig. 424. Inside surface of chicken eggshells, treated with an alcoholic solution of methyl red. (Redrawn with modifications after Glaser and Piehler, 1934.)

A, an unincubated egg; B, an egg incubated in fixed position for 20 days, showing erosion grooves left by allantoic blood vessels. Both  $\times 4$ .

The chorioallantoic veins contain redder blood than the arteries (Baer, 1828b, p. 124; Towne, 1839; Vulpian, 1857b) and thus reveal their function of carrying oxygen to the embryo. After pulmonary respiration begins, the allantoic circulation becomes less active, and, during the chick's last 2 days of incubation, many vessels in the membrane are empty (Vulpian, 1857b). On the day before the duck (*Anas platyrhynchos*) embryo hatches, blood is flowing in only the most proximal portions of the umbilical arteries and vein (Kutsuna, 1933).

The veins of the inner allantoic limb possess a layer of circular muscle which endows them with contractility (Vulpian, 1857b). Vulpian (1857b), experimenting with 15- and 16-day chick embryos, found that the umbilical vein, when stimulated with a galvanic current, contracted to one fifth or one sixth of its usual diameter at the points of contact of the poles. The response was delayed for half a minute after stimulation and reached its



maximum 4 or 5 minutes later. Small venous branches contracted so strongly that they were obliterated. Vulpian (1857*b*) also reported that neither the chorioallantoic vessels nor the arteries of the inner limb responded to electrical stimuli, although he obtained a single response (dilatation) to mechanical stimulation of an artery.

### *The Capillaries*

Early in the chick's fourth day of incubation, the mesodermal portion of the allantoic sac is traversed by a coarse vascular network. The differentiation of the characteristic respiratory capillary plexus of the chorioallantois begins a day later, as soon as the allantois starts to fuse with the chorion. Contact between the two membranes is essential for this process, which does not take place when the allantois is so stunted by experimental interruption of the Wolffian ducts that it fails to reach the chorion (Boyden, 1924).

In the 6-day chick embryo's chorioallantois, the capillaries are located immediately beneath the ectoderm which delimits the membrane externally (Fülleborn, 1895). By the tenth day, the terminal capillaries have begun to push farther out toward the eggshell and are now found between the inner and outer layers of ectodermal cells (Fig. 425-A). After 13 to 15 days of incubation (Fig. 425-B), many of these vessels have penetrated completely through the ectoderm and have expanded above it (Danchakoff, 1917). They thus lie directly under the shell membranes, with no living tissue intervening between them and the external atmosphere. The ready access to oxygen probably accounts for the ability of allantoic tissue to survive overheating longer than any other tissue in the incubating egg. As shown by the capacity to regenerate when cultured, both limbs of the 10-day chick embryo's allantoic sac can withstand a temperature of 45.5° C. for 11 to 15 hours (Szarski, 1948).

The capillary network of the chorioallantois is so fine and so dense that it is comparable only to the capillary plexuses found in the chorioid of the eye and the lungs of higher vertebrates. The vessels, although they are wider than the interspaces between them (Fülleborn, 1895), are of such small caliber that the blood cells are forced to pass through them in single file. The sudden dispersion of the blood into this vascular bed causes a slackening in the speed of the blood stream, a circumstance which is essential for gaseous exchange. The discontinuous movement of the blood corpuscles through the chorioallantoic plexus also characterizes the latter as respiratory in nature. The blood cells are propelled out of the small arterioles by rhythmic pulsations and proceed through the capillaries jerkily until, entering somewhat larger vessels that converge upon the vein roots, they gather speed and pass into the veins rapidly and smoothly (Giacomini, 1930*a*, 1930*b*). Experiments have shown, furthermore, that the structure of



the network is responsive to the concentration of oxygen in the atmosphere. On the chick's third or fourth incubation day, Remotti (1933*d*) increased the partial oxygen pressure of the air in the incubator to 50 to 75 per cent of the total. By the sixth day, it could be seen that the individual chorioallantoic capillaries were of wider caliber than normal and arranged in larger meshes. This reduction in the vascular surface available for gaseous

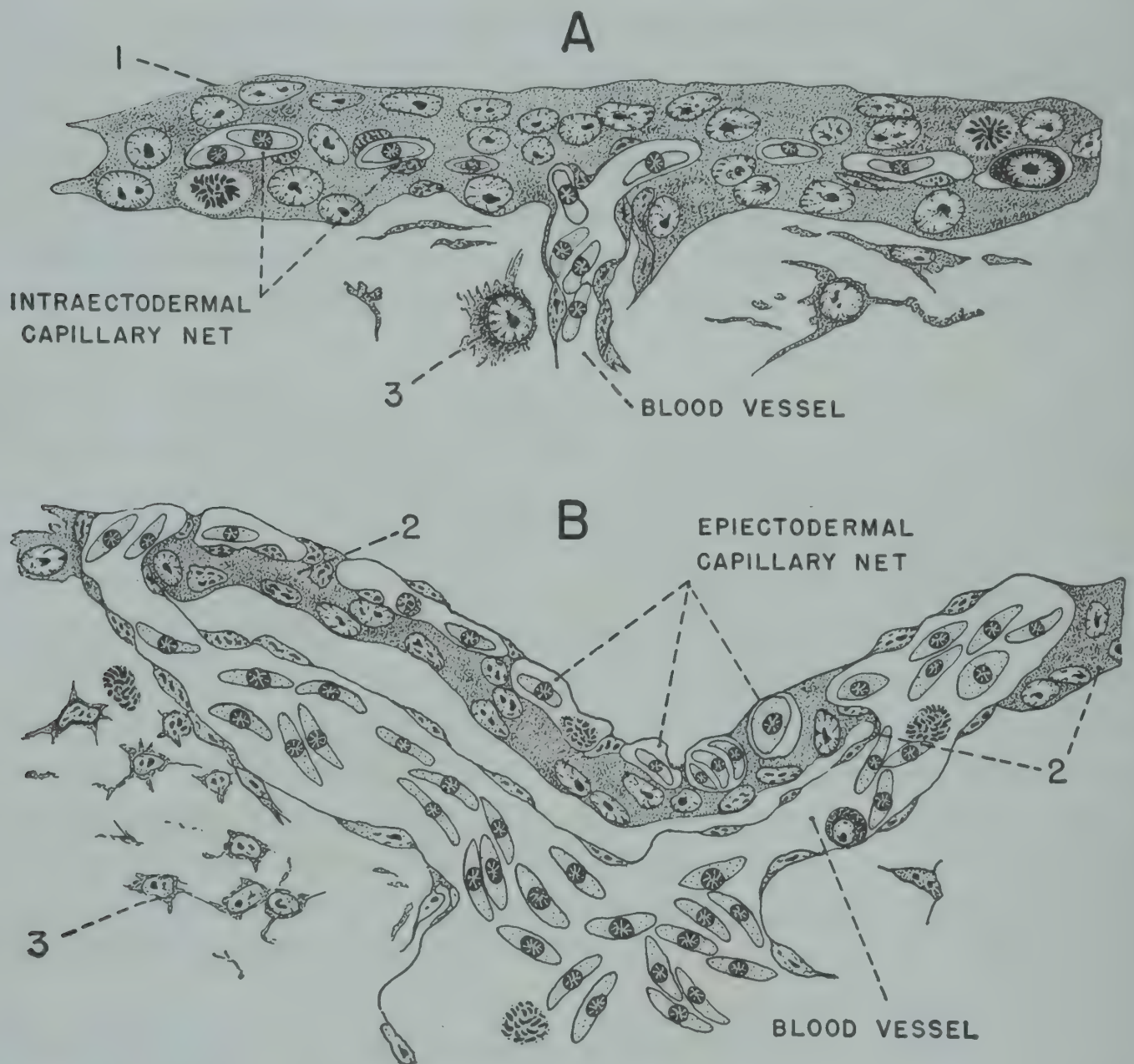


Fig. 425. Cross sections of the superficial layer of the outer wall of the allantois of the chick showing two successive positions of the capillary net in relation to the ectoderm. (Redrawn with modifications after Danchakoff, 1917.)

A, a section from a 10-day embryo; B, a section from a 15-day embryo. Both  $\times 400$ . 1, ectoderm of chorioallantois; 2, subvascular ectoderm; 3, mesenchyme.

exchange appears to be compensatory for the greater diffusion of oxygen. Similar changes occur when the atmospheric pressure is increased (*Flemister and Cunningham, 1940*). Modification of structure in relation to respiratory function has also been shown by experiments in which all the eggshell was covered with impermeable varnish except for a portion that was removed completely. After incubation in an atmosphere enriched in oxygen, peculiar systems of spiral capillaries developed in the part of the chorioallantois



directly exposed to the air, whereas in the area deprived of oxygen the connections between the arterioles and venules were shorter and simpler than normal (*Remotti, 1935a*).

At the outer edge of the chorioallantois, the capillary plexus makes the transition to the luxuriant but wider-meshed vascular network of the inner allantoic limb. Around the periphery of the region of amnioallantoic fusion, the capillaries of the inner allantoic limb, like the muscle bundles, are parallel to each other and perpendicular to the circumference of the fused area. The vessels in the midst of the area of fusion do not persist as long as those around its edge and are already beginning to atrophy on the chick's nineteenth incubation day (*Fülleborn, 1895*).

### *Anastomoses with Other Vascular Systems*

The vessels of the inner allantoic limb eventually form anastomoses with those of the amnion and the yolk sac. Although the fusion of amnion and allantois begins on the chick's seventh incubation day, the amnion—according to Baer (*1828b, p. 124*)—does not receive its blood supply until the eleventh day; therefore the first anastomoses between amniotic and allantoic vessels occur after this time. In the coot embryo (*Fullica atra*), they have formed by the fifteenth day (*Steinmetz, 1930*). Fülleborn (*1895*) stated that anastomoses take place only in a limited region.

The anastomoses between the allantoic and the vitelline vascular systems form in the vicinity of the yolk sac umbilicus. During or somewhat before the chick's twelfth day of development, vessels originating in the yolk sac begin to invade the portion of the chorion which is contiguous to the yolk sac umbilicus and has not yet come into contact with the allantois. Spampani (*1905*) reported this occurrence in the 18-day embryo of the sparrow (*Passer d. italiae*). On the chick's thirteenth day, this part of the chorion begins to be vascularized by capillaries of the inner allantoic limb. The nonvascularized region between the two vessel areas becomes continually smaller as the albumen sac decreases in size, until the capillaries of allantoic and vitelline origin finally meet about on the sixteenth day (*Fülleborn, 1895*). Popoff (*1894*) was able to inject allantoic blood vessels from the yolk sac on the nineteenth day, and Fülleborn (*1895*) showed that yolk sac vessels can be filled by injections into the allantoic circulation on the seventeenth day.

The anastomoses formed by the allantoic blood vessels establish secondary interconnections among all the vascular systems found within the incubating egg. Thus the anastomoses with the amniotic vessels connect the allantoic and embryonic circulations; and the anastomoses with the yolk sac vessels not only connect the yolk sac circulation with that of the albumen sac but in turn connect these circulations with that of the embryonic body and the amnion (*Steinmetz, 1930*).



*The Lymphatic Vessels*

All portions of the allantois contain very abundant lymphatic vessels which accompany the blood vessels and are especially well developed around the arteries. As in the embryo's body, the lymphatics are established from endothelial buds; these can be seen protruding into the allantoic cavity as papilliform excrescences. The allantoic lymphatic system is probably present on the chick's sixth day of incubation, but it cannot be seen clearly until the seventh or eighth day (*Fülleborn, 1895; Creighton, 1899; Boyden, 1929*); at this time, it can be injected (*Budge, 1887*), as it can in the 9-day duck (*Anas platyrhynchos*) embryo (*Kutsuna, 1933*).

The lymphatics of the allantois are already present in great luxuriance when they are first demonstrable by injection. All the blood vessels, large and small, are not only accompanied in their course by lymphatic vessels, but—with the exception of the capillaries—are surrounded by virtual "sheaths" of cross-anastomoses connecting the accompanying lymphatics. As a result, the smallest lymphatics form a very fine and dense capillary network, densest around the largest blood vessels (*Kutsuna, 1933*). The lymphatic stems accompanying the veins are of somewhat smaller caliber than those associated with the arteries (*Fülleborn, 1895*). As incubation continues, the lymphatics increase in size, and the periarterial meshes become elongated in shape (*Kutsuna, 1933*); also, some differences appear in the distribution of the lymphatics in the inner and outer allantoic limbs. In the chorioallantois, where the lymphatic system is better developed than in the inner limb, even the smaller vascular branches each possess two accompanying lymphatic stems connected by cross-anastomoses. A terminal network of lymphatic capillaries forms directly beneath the blood capillary network. In the inner limb, arteries with accompanying veins may be escorted by two lymphatic vessels, one beside the artery and the other beside the vein, and these lymphatic vessels are connected by anastomoses which surround the paired blood vessels (*Fülleborn, 1895*).

The allantoic lymphatics drain into the posterior lymph hearts and the thoracic ducts (*Budge, 1882; Sala, 1900; Mierzejewski, 1909; Clark, 1915a*). *Kutsuna (1933)* described the drainage of lymph from the allantois in duck (*Anas platyrhynchos*) embryos of various ages. In the allantoic stalk of the 10-day embryo, the umbilical artery and vein are surrounded by fine, intercommunicating lymphatics which follow the artery into the embryonic body and accompany its branches to right and left. On each side, these lymphatics then empty into the developing posterior lymph heart and into the plexiform caudal end of the thoracic duct. At the 13-day stage, the lymphatics associated with the umbilical artery have formed a single large vessel which communicates with the posterior lymph heart by a short canal



and, the next day, is connected with the thoracic duct by means of the large lymphatic sac in the iliac region.

The allantoic lymphatics undergo involution during the latter part of the incubation period. A lymphatic vessel of wide caliber can still be seen, however, in the allantoic stalk of the duck (*Anas platyrhynchos*) on the day before hatching (*Kutsuna, 1933*).

### The Allantoic Fluid

Before it receives the excretions of the embryonic kidneys, the allantoic sac contains a clear, colorless liquid which is probably derived from its own

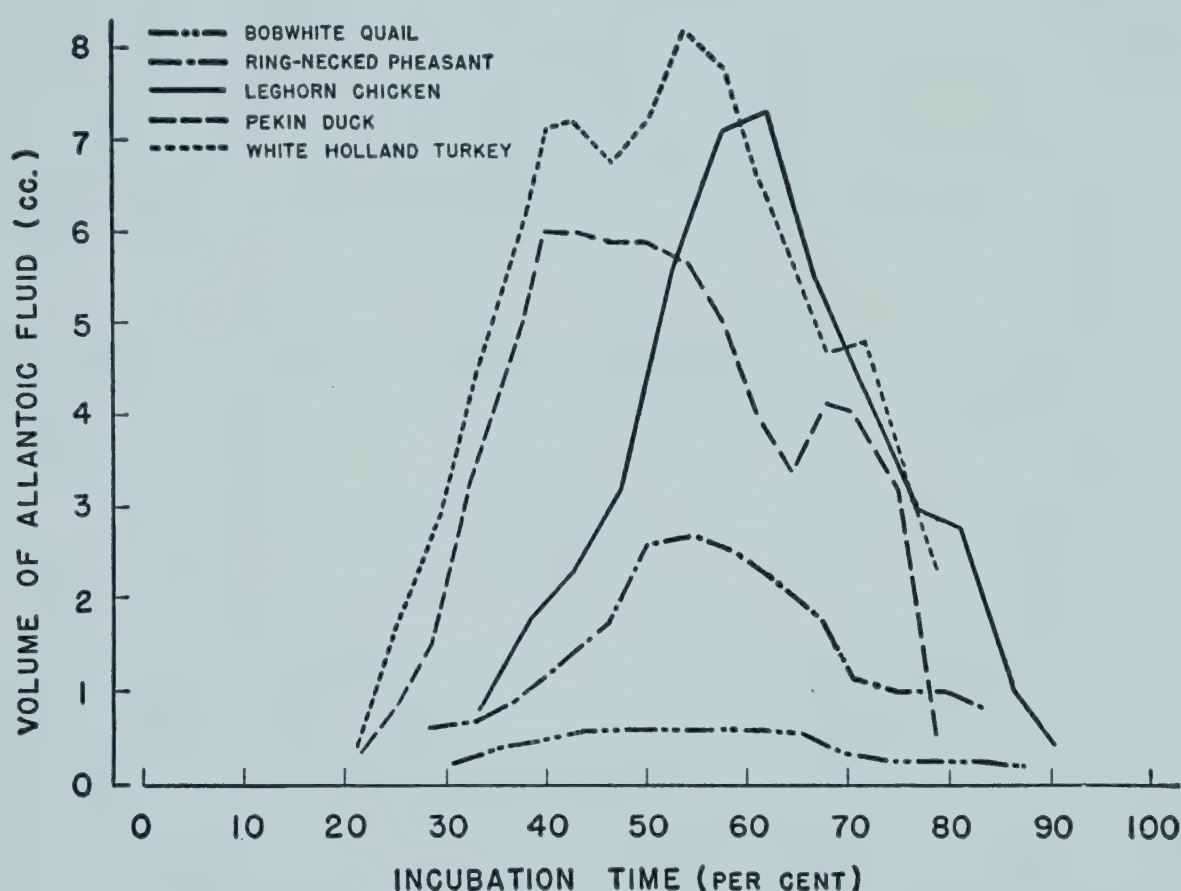


Fig. 426. Changes in volume of allantoic fluid during incubation of the bobwhite quail, *Colinus virginianus*, the ring-necked pheasant, *Phasianus colchicus*, the Leghorn chicken, *Gallus gallus*, the Pekin duck, *Anas platyrhynchos*, and the White Holland turkey, *Meleagris gallopavo*. (Plotted after the data of Romanoff and Hayward, 1943.)

walls or from the cloaca (*Boyden, 1929*). Early in the chick's fourth day of incubation, urine begins to flow into the allantois, and it continues to do so throughout the remainder of the incubation period.

After the eleventh day, the allantoic fluid has a yellowish tinge (*Fiske and Boyden, 1926*). At some time between the seventh and fourteenth days (*Vulpian, 1857b; Fiske and Boyden, 1926*), the concentration of waste products (urates) becomes sufficiently high to give the allantoic fluid a cloudy appearance. During this period, some of the urates become coated with what is apparently mucus and begin to coalesce into flat plaques and to be deposited in solid form (*Fiske and Boyden, 1926*).



The fluid accumulates rapidly until, on the thirteenth day, it reaches its maximum volume, usually 6 to 7 cc. (*Fiske and Boyden, 1926; Romanoff, Smith, and Sullivan, 1938; Romanoff and Hayward, 1943; Kugler, 1945*), but occasionally as much as 10 cc. (*Randles, 1951*) or as little as 4 cc. (*Yamada, 1933*). As Fig. 426 shows, the largest amount of fluid has been found at a comparable time (*Romanoff and Hayward, 1943*) in the incubation periods of the pheasant (*Phasianus colchicus*), the bobwhite quail (*Colinus virginianus*), the turkey (*Meleagris gallopavo*), and the duck (*Anas platyrhynchos*). Experiments with the chick embryo have shown that the maximum quantity of fluid is less than normal when the incubation temperature is above or below 37.5° C. Also, the fluid accumulates more rapidly at elevated temperatures and more slowly at subnormal temperatures, so that the peak in volume occurs earlier in the one case and later in the other (*Romanoff, Smith, and Sullivan, 1938; Romanoff and Hayward, 1943*). The accompanying tabulation indicates the magnitude of these changes.

Incubation Temperature (° C.)	Maximum Volume of Fluid (cc.)	Incubation Age When Volume Is Maximum (days)
34.5	1.50	14
37.5	6.15	13
39.5	1.95	11

Because of the absorption of water (*Boyden, 1929*), the allantoic fluid rapidly diminishes in amount during the chick's fourteenth to nineteenth incubation days; practically none of it remains by the twentieth day (*Vulpian, 1857b; Yamada, 1933; Romanoff, Smith, and Sullivan, 1938*). The allantoic cavity is now filled with a mass of solid deposits (*Fiske and Boyden, 1926*). At incubation temperatures above 37.5° C., or at 34.5° C., the fluid, already of less than normal volume, disappears sooner. At a moderately lowered incubation temperature (35.5° C.), the fluid is present in almost normal amount but may persist in measurable quantity until the twenty-first day (*Romanoff, Smith, and Sullivan, 1938*).



# APPENDIX



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- Weight of the avian embryo (Table I).
- Absolute and proportional weights of body parts of the chicken embryo (Table II).
- Variations in weight of the chicken embryo (Figure I).
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- Length of limbs of the chicken embryo (Table III).
- Influence of temperature on the weight of the chicken embryo (Table IV).
- Influence of size of the egg on the weight of the chicken embryo (Table V).
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Average Weight of the Developing Avian Embryo

Age of Embryo	Chicken ( <i>Gallus gallus</i> )	Jungle Fowl ( <i>Gallus gallus</i> )	Ring-Necked Pheasant ( <i>Phasianus colchicus</i> )	Bobwhite Quail ( <i>Colinus virginianus</i> )	Turkey ( <i>Meleagris gallopavo</i> )	Indian Runner ( <i>Anas platyrhynchos</i> )	Embden Goose ( <i>Anser anser</i> )	Pigeon ( <i>Columba livia</i> )	Gray Crow ( <i>Corvus cornix</i> )
(days)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
1	0.0002								
2	0.003								0.020
3	0.021				0.011	0.005	0.011	0.008	0.220
4	0.060	0.058	0.008	0.004	0.036	0.056			
5	0.160		0.027	0.021	0.089	0.140		0.130	0.650
6	0.34	0.09	0.099	0.044	0.25	0.21	0.202	0.43	
7	0.64	0.62	0.22	0.13	0.40	0.41			0.95
8	1.07	1.10	0.41	0.24	0.75	0.76			
9	1.56	1.73	0.76	0.38	1.68	1.19	1.006	1.08	2.30
10	2.39	2.37	1.02	0.46	1.88	1.91			
11	3.49	3.30	1.45	0.70	2.97	2.67	2.85	2.21	3.50
12	5.04	4.38	1.84	0.83	3.19	3.71			
13	7.05		2.81	1.05	4.48	4.86		3.86	6.70
14	10.16	7.24	3.37	1.30	5.48	7.04			
15	12.50		4.40	1.65	7.07	9.53	6.82	7.07	13.70
16	14.60		5.72	1.85	10.27	14.14			
17	18.31	9.70	6.70	2.33	13.01	16.11		9.72	16.00
18	22.09	14.05	8.09	2.80	17.87	19.37	18.38		
19	25.59	15.04	10.05	3.48	19.60	22.34			
20	29.71	15.57	11.53	3.61	20.73	26.45			
21	30.97	17.64	11.65	4.25	28.17	28.78	44.21		
22		23.72	12.31	4.68	32.52	33.28			
23			14.94	5.08	38.22	39.83			
24			16.34	5.57	39.81	40.66	63.85		
25			17.99	6.43	44.40	43.58			
26					46.96	45.77			
27					49.36	47.87	84.16		
28									
29									
30							93.66		

Average Egg Weight	60	36	32	9	80	65	120	17	30
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Chicken (Leghorn) : after Schmalhausen (1926) and Romanoff (1928 ; unpublished) ; jungle fowl, ring-necked pheasant, bobwhite quail, turkey, duck, and goose : after Romanoff (unpublished).  
Pigeon : after Kaufman (1929a, 1929b).  
Crow : after Chmutova (1949).



TABLE II  
Absolute and Proportional Weights of Various Body Parts of Leghorn Chicken (*Gallus gallus*) Embryo \*

Age of Embryo	Weight of Embryo	Head		Trunk		Neck		Hindlimb		Forelimb	
		Weight	Proportion †	Weight	Proportion	Weight	Proportion	Weight	Proportion	Weight	Proportion
(days)	(grams)	(grams)	(per cent)	(grams)	(per cent)	(grams)	(per cent)	(grams)	(per cent)	(grams)	(per cent)
4	0.11	0.07	63.1	0.03	29.7	0.01	6.3	0.0001	0.5	0.0004	
5	0.31	0.20	64.2	0.09	28.7	0.02	6.4	0.002	0.8	0.001	
6	0.54	0.29	53.8	0.16	29.7	0.03	5.6	0.007	1.0	0.003	0.1
7	1.17	0.67	57.2	0.44	37.8	0.04	3.4	0.015	1.2	0.006	0.4
8	1.52	0.84	55.1	0.60	39.5	0.05	3.3	0.024	1.5	0.010	0.6
9	2.49	1.18	47.5	1.08	43.4	0.17	6.7	0.044	1.8	0.016	0.6
10	3.53	1.40	39.7	1.75	49.6	0.23	6.5	0.118	3.2	0.035	1.0
11	5.14	1.80	35.0	2.74	53.3	0.37	7.2	0.18	3.4	0.06	1.1
12	7.79	2.25	28.9	4.55	58.3	0.70	9.0	0.22	2.8	0.08	1.0
13	8.87	2.45	27.6	5.03	56.7	0.75	8.4	0.46	5.2	0.18	2.1
14	10.80	2.85	26.4	6.02	55.7	0.95	8.8	0.71	6.6	0.27	2.5
15	16.85	3.26	19.3	10.73	63.8	1.57	9.3	0.95	5.6	0.34	2.0
16	19.70	3.75	19.0	12.50	63.5	1.90	9.6	1.15	5.8	0.40	2.1
17	23.60	4.15	17.6	15.13	64.1	2.20	9.3	1.60	6.8	0.52	2.2
18	26.14	4.40	16.8	16.44	62.9	2.35	9.0	2.30	8.8	0.65	2.5
19	28.13	4.60	16.3	17.51	62.3	2.61	9.3	2.60	9.2	0.81	2.9
20	29.77	4.77	16.0	18.67	62.7	2.64	8.9	2.73	9.2	0.96	3.2
21	31.27	4.99	15.9	19.60	62.7	2.82	9.0	2.80	9.0	1.06	3.4

\* Summarized after Schmalhausen (1927); Kuo (1932d).

† Calculated as  $\frac{\text{Body Part}}{\text{Whole Embryo}} \times 100$ .



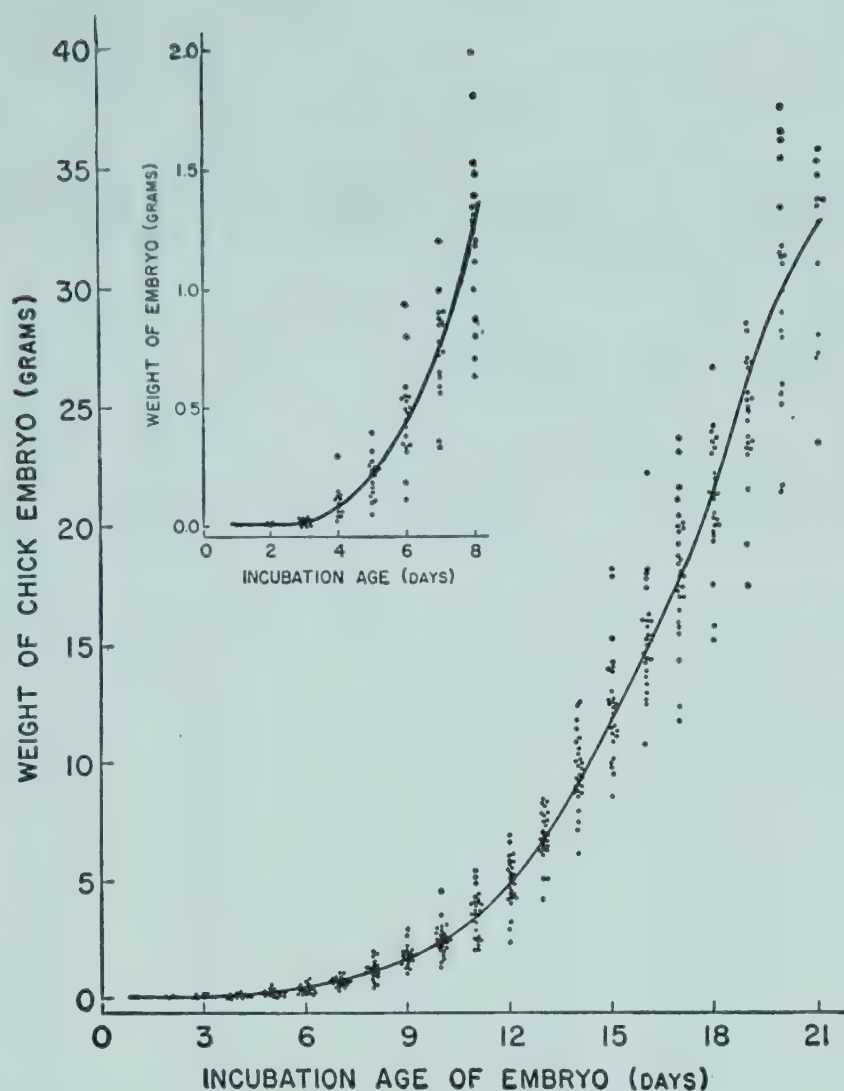


Fig. I. Daily weight of the chicken embryo of the egg-producing breeds of *Gallus gallus*, as measured by twenty-eight different investigations throughout the world. (Compiled by the author.)

Each dot represents an average value for the set of observed embryos. This chart, together with its *Insert*, demonstrates the possible deviations in the growth of the embryo, presumably due to variations in environmental conditions of incubation.

INCUBATION AGE	RELATIVE SIZE OF CHICK EMBRYO AT VARIOUS TEMPERATURES							
	29.5° C.	31.5° C.	33.5° C.	35.5° C.	37.5° C.	39.5° C.	41.5° C.	43.5° C.
12 HOURS								
24 HOURS								
48 HOURS								
72 HOURS								
96 HOURS								all dead

Fig. II. Increase in size of embryonal area of the early chick (*Gallus gallus*) at various incubation temperatures. (Measured from mounted embryos after Romanoff, unpublished.)



TABLE III

Average Length of Various Parts of Forelimb and Hindlimb of the  
Developing Chicken (*Gallus gallus*) Embryo

Age of Embryo (days)	Length of Hindlimb *			Length of Forelimb †		
	Femur	Tibia	Tarsus	Humerus	Ulna	Radius
	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)	(mm.)
5	0.2	0.7		0.6	0.5	0.5
6	1.2	1.3		1.0	1.0	0.8
7	2.3	2.7	1.7	2.3	2.0	1.7
8	4.1	4.5	2.7	3.4	3.0	2.8
9	5.3	6.2	4.7	4.3	4.1	3.8
10	6.4	8.3	5.8	5.0	4.8	4.5
11	7.9	10.3	6.8	5.9	5.6	5.3
12	8.8	12.0	7.8	6.8	6.3	6.0
13	9.5	13.5	9.1	7.8	7.2	6.8
14	11.5	15.4	11.1	8.6	8.3	7.4
15	13.3	18.2	12.8	9.2	8.5	7.9
16	14.7	20.4	14.7	9.9	8.8	8.3
17	16.0	23.4	17.2	10.8	9.8	9.0
18	18.1	25.5	18.2	11.3	10.3	9.6
19	19.3	27.4	19.7	12.5	11.5	11.0
20	20.3	28.5	21.3	13.1	11.9	11.3
21	23.0	32.0	23.8	15.3	14.1	13.1

\* Averaged after Stepanova (1926); Landauer (1939); Haardick (1941).

† Averaged after Stepanova (1926); Landauer (1939).



TABLE IV

Average Weight of Leghorn Chicken (*Gallus gallus*) Embryo as Influenced by Incubation Temperature \*

Age of Embryo	Incubation Temperature ° C.							
	33.5	34.5	35.5	36.5	37.5 †	38.5	39.5	40.5
(days)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
3		0.006	0.008	0.016	0.018	0.023	0.034	0.043
4	0.007	0.015	0.032	0.036	0.059	0.082	0.098	0.105
5	0.019	0.049	0.072	0.115	0.143	0.215	0.312	0.248
6	0.05	0.07	0.21	0.30	0.37	0.45	0.68	0.59
7	0.08	0.19	0.31	0.63	0.69	0.93	1.06	1.01
8	0.20	0.31	0.63	0.94	1.15	1.34	1.62	1.66
9	0.33	0.60	0.95	1.56	1.66	2.00	2.27	2.49
10	0.47	0.77	1.29	1.94	2.28	2.72	3.20	3.90
11	0.70	1.07	1.90	2.60	3.39	4.03	4.30	5.09
12	0.87	1.59	2.30	3.75	5.22	7.13	7.21	5.59
13	1.62	1.88	3.21	5.81	7.31	9.32	8.76	7.09
14	Dead	3.00	5.26	8.47	10.99	10.92	9.71	Dead
15		3.62	6.89	10.59	13.12	13.57	11.02	
16		5.10	9.05	15.52	16.63	16.47	14.85	
17		6.17	11.24	18.63	19.62	20.96	18.50	
18		7.92	13.76	19.49	22.18	22.94	20.45	
19		9.18	17.44	24.90	27.03	28.52	22.14	
20		Dead	19.25	31.05	31.75	31.95	Hatched	
21			21.91	32.92	33.44	Hatched		
22			23.46	33.53	Hatched			
23			27.48	34.45				
24			33.61	Hatched				
25			Hatched					

\* From the data of Romanoff, Smith, and Sullivan (1938). The average size of the egg was  $60 \pm 2$  grams.

† Physiologically normal incubation temperature.



TABLE V  
Average Weight of Chicken (*Gallus gallus*) Embryo as  
Influenced by the Size of the Egg \*

Age of Embryo	Weight of Freshly Laid Eggs in Grams				
	30	40	50	60	70
	Weight of Developing Embryo				
(days)	(grams)	(grams)	(grams)	(grams)	(grams)
1				0.0008	
2	0.0008			0.005	
3	0.028	0.021	0.020	0.033	
4	0.09	0.11	0.10	0.13	
5	0.20	0.33	0.17	0.27	
6	0.51	0.61	0.33	0.57	
7	0.78	0.89	0.67	0.96	
8	1.13	1.19	1.06	1.45	
9	1.84	1.82	1.49	2.01	
10	2.63	2.45	2.77	3.15	
11	2.92	3.08	3.52	4.31	3.33
12	4.67	5.62	4.92	6.10	4.53
13	5.92	8.15	7.97	8.38	6.93
14	8.20	10.68	10.82	12.01	10.43
15	10.07	13.22	14.07	14.45	13.24
16	11.02	16.19	14.56	17.69	16.55
17	13.93	16.73	18.82	21.09	20.36
18	14.82	20.30	22.48	23.88	24.60
19	16.29	22.10	25.61	28.77	29.11
20	17.07	26.20	26.63	33.94	35.88
21	18.78	28.82	30.99	35.60	38.44

\* Compiled from the data of Byerly (1932) and Romanoff (*unpublished*). The eggs were incubated at the optimum temperature, about 37.5°C.



TABLE VI

Average Weight of Various Organs of the Chicken (*Gallus gallus*) Embryo

Age of Embryo	Brain	Eye	Eyeball	Lungs	Heart	Liver	Gizzard	Primordial Kidney (Meso- nephros)		Definitive Kidney (Meta- nephros)		Spleen	Intestines
								(grams)	(grams)	(grams)	(grams)		
(days)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
2	0.0004	0.00001		0.000001									
3	0.0025	0.00009		0.000013	0.00014	0.00003							
4	0.010	0.0007	0.000006	0.000046	0.00071	0.00030	0.0001	0.0002	0.0002	0.0002			
5	0.038	0.009	0.00017	0.000153	0.00231	0.00152	0.0005	0.0007	0.0006	0.0006			
6	0.063	0.027	0.00032	0.00032	0.0046	0.0047	0.0011	0.0015	0.0013	0.0013			
7	0.083	0.068	0.00059	0.00186	0.0075	0.0053	0.0029	0.0040	0.0028	0.0028		0.00005	0.0095
8	0.107	0.112	0.00091	0.0054	0.0107	0.0175	0.0058	0.0065	0.0044	0.0044		0.00016	(0.0121)
9	0.133	0.150	0.00123	0.0094	0.0145	0.0274	0.0119	0.0093	0.0061	0.0061		0.00048	0.0387
10	0.166	0.202	0.00177	0.0185	0.0212	0.0451	0.0237	0.0116	0.0107	0.0107		0.00088	(0.0618)
11	0.227	0.220	0.00204	0.026	0.030	0.070	0.040	0.014	0.013	0.013		0.0013	0.100
12	0.262	0.238	0.00252	0.037	0.039	0.109	0.067	0.016	0.019	0.019		0.0022	(0.215)
13	0.338	0.265	0.00310	0.055	0.053	0.154	0.135	0.019	0.033	0.033		0.0033	0.330
14	0.414	0.285	0.00355	0.064	0.072	0.215	0.190	0.021	0.048	0.048		0.0046	(0.514)
15	0.500	0.310	0.0040	0.074	0.075	0.258	0.275	0.020	0.054	0.054		0.0050	0.697
16	0.610	0.335	0.0050	0.094	0.110	0.397	0.394	0.018	0.077	0.077		0.0057	(0.967)
17	0.687	0.362	0.0062	0.121	0.136	0.479	0.534	0.015	0.094	0.094		0.0060	1.244
18	0.775	0.400	0.0074	0.141	0.156	0.535	0.766	0.009	0.104	0.104		0.0063	(1.593)
19	0.889	0.420	0.0080	0.161	0.163	0.658	0.992	0.007	0.116	0.116		0.0065	1.951
20	0.924	0.450	0.0090	0.212	0.189	0.730	1.242	0.006	0.137	0.137		0.0068	(2.433)
21	0.985	0.470	0.0100	0.287	0.310	1.009	1.699	0.005	0.190	0.190		0.0070	2.916

Brain: Schmalhausen (1927); Duyff (1939); Yushok (1950).

Eye: Schmalhausen (1927); Landauer (1939).

Eyeball: Schmalhausen (1927).

Lungs: Schmalhausen (1927); Kaufman (1929b); Romanoff (unpublished).

Heart: Schmalhausen (1927); Kaufman (1929b); Olivo (1930); Romanoff (1933); Duyff (1939); Landauer (1939); Yushok (1950); Hermann and Barry (1955).

Liver: Schmalhausen (1927); Kaufman (1929b); Romanoff, Smith, and Sullivan (1938); Duyff (1939); Landauer (1939); Yushok (1950); Hermann and Barry (1955); Romanoff and Laufer (1956).

Gizzard: Schmalhausen (1927); Landauer (1939); Kuo and Shen (1936).

Primordial Kidney (mesonephros): Schmalhausen (1927); Kaufman (1929b).

Definitive Kidney (metanephros): Schmalhausen (1927); Kaufman (1929b).

Spleen: Schmalhausen (1927); Landauer (1939).

Intestines: Kaufman (1929b).



TABLE VII  
Average Weight of Various Organs of the Pigeon (*Columba livia*) Embryo \*

Age of Embryo	Brain	Eye	Lungs	Heart	Liver	Primordial Kidney (mesonephros)	Definitive Kidney (metanephros)	Intestines
(days)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
5		0.003		0.002	0.001			
7	0.027	0.023	0.003	0.005	0.006	0.004		0.006
9	0.072	0.081	0.020	0.009	0.016	0.005	0.003	0.019
11	0.114	0.145	0.071	0.017	0.038	0.006	0.013	0.085
13	0.163	0.182	0.090	0.033	0.074	0.007	0.037	0.237
15	0.225	0.251	0.133	0.058	0.163	0.005	0.094	0.580
17	0.302	0.255	0.132	0.074	0.249	0.002	0.129	0.763

\* After Kaufman (1926, 1929b).



TABLE VIII

Average Weight of Various Endocrine Glands of the Chicken  
(*Gallus gallus*) Embryo

Age of Embryo	Thyroid		Adrenals		Thymus		Hypophysis		Ovary (left)		Testicles	
	Ref. 1	(mg.)	Ref. 1	(mg.)	Ref. 1	(mg.)	Ref. 1	(mg.)	Ref. 1	(mg.)	Ref. 1	(mg.)
(days)	Ref. 1	(mg.)	Ref. 2	(mg.)	Ref. 3	(mg.)	Ref. 4	(mg.)	Ref. 4	(mg.)	Ref. 4	(mg.)
6												0.05
7												0.15
8												0.25
9												0.28
10	0.315		0.800		1.01	3.42	0.30		0.72		0.58	0.35
11	0.420		1.20		1.60		0.37		0.92		0.68	0.42
12	0.665		1.53	0.750	4.18	4.66	0.50		2.02		0.73	0.52
13	1.22		2.06	1.12	8.34		0.86		2.93		1.22	0.68
14	1.31		2.29	1.55	12.35	7.03	0.90		3.95		1.53	0.80
15	2.56		3.56	2.08	20.50		1.00		4.60		3.60	1.10
16	3.96		4.70	3.19	37.90	8.69	1.42		5.33		5.36	1.80
17	4.49		5.00	3.30	48.60		1.45		7.80		6.38	2.20
18	5.14		5.36	4.50	58.54	26.18	1.44		8.00		6.33	3.20
19	6.16		6.16	4.76	71.95		1.42		7.85		7.08	4.00
20	7.20		7.20	6.00	109.68		1.42		7.96		7.33	
21	7.16		7.96	6.38	101.33	37.53	1.45		8.20		7.50	
21 *	3.70(109)		6.10(104)						5.60(41)		6.10(61)	

1. Venzke (1943); 2. Case (1952); 3. Dmitrieva (1939); 4. Schmalhausen (1927).  
\* Romanoff and Laufer (1956). Figures in parenthesis indicate number of observations.







## BIBLIOGRAPHY



*When books are amply documented,  
With history in every part,  
Their contents are well represented  
By facts of those who loved their art.*



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# INDEX



*A subject index has objectives:*

*To fill the gaps in searchers' thought,  
And offers them exact directives  
To places, facts which they have sought.*



# INDEX

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